



THE EFFECTIVENESS OF EVALUATION OF PROTEIN-FERMENTED PRODUCTS' USE IN BROILER CHICKENS FEED

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AIM OF THE RESEARCH on chickens

The aim of the study is to determine whether the addition of fermented protein products (rapeseed/soybean meals) to the chickens' feed mixture, can improve the productivity and immunity of chickens having the positive impact on the histology of intestine and microflora from digestive tract.



MATERIALS AND METHODS

Experimental design

- The material for the study consisted of one day-old COOB chicks raised until their 42nd day of age
- The experiment was carried out on 5000 chicks assigned to five experimental groups of 1000 birds each
- The control group (G-C) did not receive fermented soybean /fermented rapeseed meals
- Groups: FS-3%, FS-6% received fermented soybean meals in their base compound feed in the amounts of 3% or 6%
- Groups: FR-3%, FR-6% received fermented rapeseed in their base compound feed in the amounts of 3% or 6%

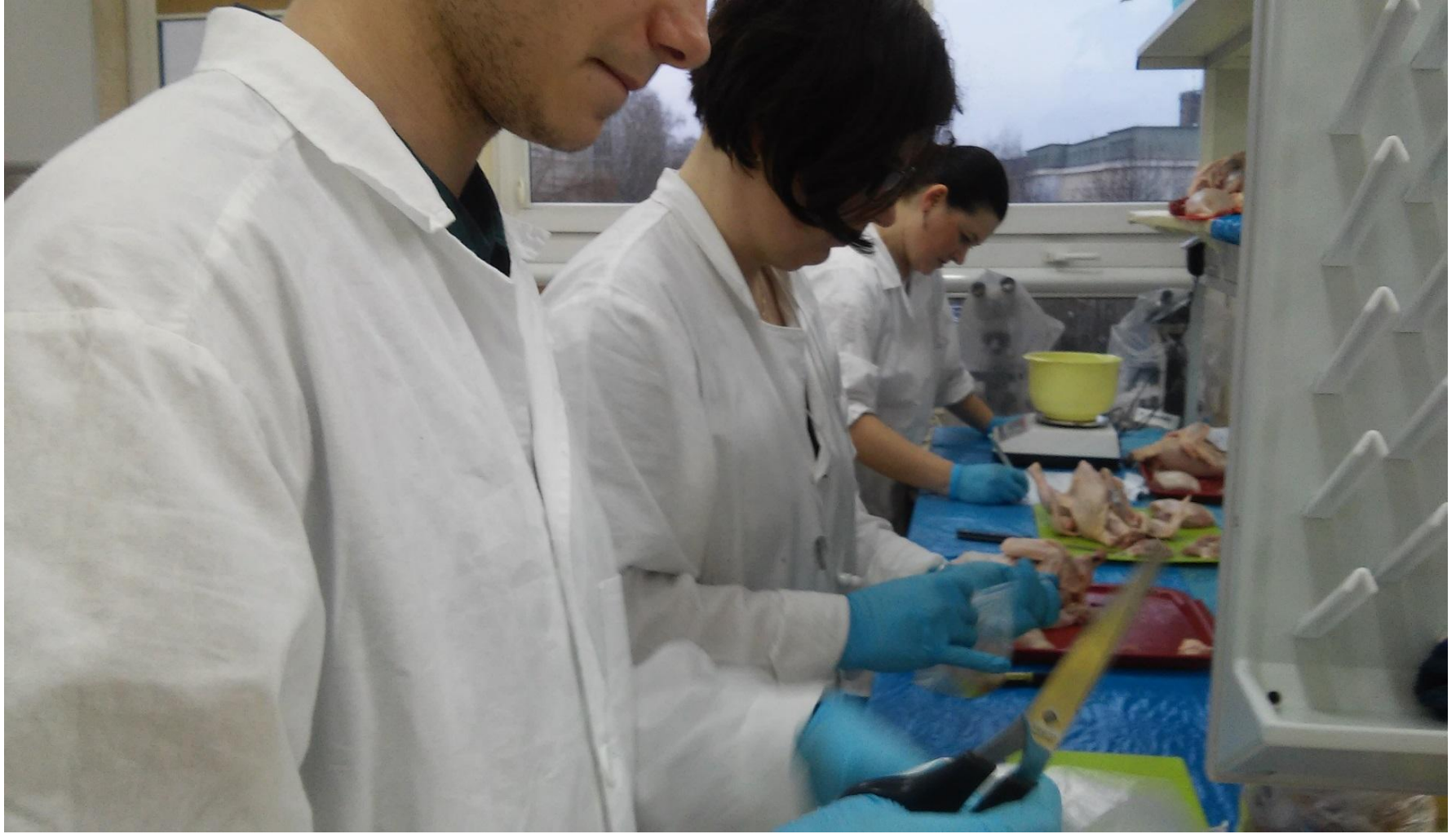
G-C	FS-3%	FS-6%	FR-3%	FR-6%
control group standard (complete) mixture feed	balanced mixture feed based on 3% of fermented soybean meals	balanced mixture feed based on 6% of fermented soybean meals	balanced mixture feed based on 3% of fermented rapeseed meals	balanced mixture feed based on 6% of fermented rapeseed meals

The chickens had permanent access to drinking water and received ad libitum complete feed mixes appropriate for the rearing period according to the Nutrient Requirements for Poultry (2005).

MATERIALS AND METHODS

Performance

- During the experiment, the body weight of the broiler chickens was monitored at the end of each week of rearing (all birds were also weighted at the start of the experiment).
- Feed intake and mortality were monitored during the entire growth period. After the rearing period **8 chickens** from each experimental **group** were slaughtered.
- The slaughtered birds were dissected and the carcasses were analysed after dissection.









Sampling the intestinal and intestinal gastric contents for microbiological analyzes





Parameters determined in biological material

The microbiological analysis of the digesta from the jejunum and droppings

- total number of aerobic mesophilic bacteria,
- the total number of yeast and moulds,
- the total number of coli group bacteria,
- the number of *Escherichia coli*,
- the number of *Lactobacillus*,
- the number of *Salmonella, Campylobacter* group bacteria



Parameters determined in biological material

The histological examination of the villi and crypts of the jejunum

The stretch of the jejunum was collected from **8 slaughtered chickens** of each group for histopathological examination. In each intestine's preparation **20 villi** (longitudinally cut out) and **20 intestinal's crypts** will be measured .



Parameters determined in biological material

Blood analysis

Haematological parameters:

- haematocrit value (HT),
- haemoglobin level (HB),
- total white blood cell (WBC),
- blood leucocyte profile (leucogram)
- indicator erythrocyte sedimentation (ESR)



Parameters determined in biological material

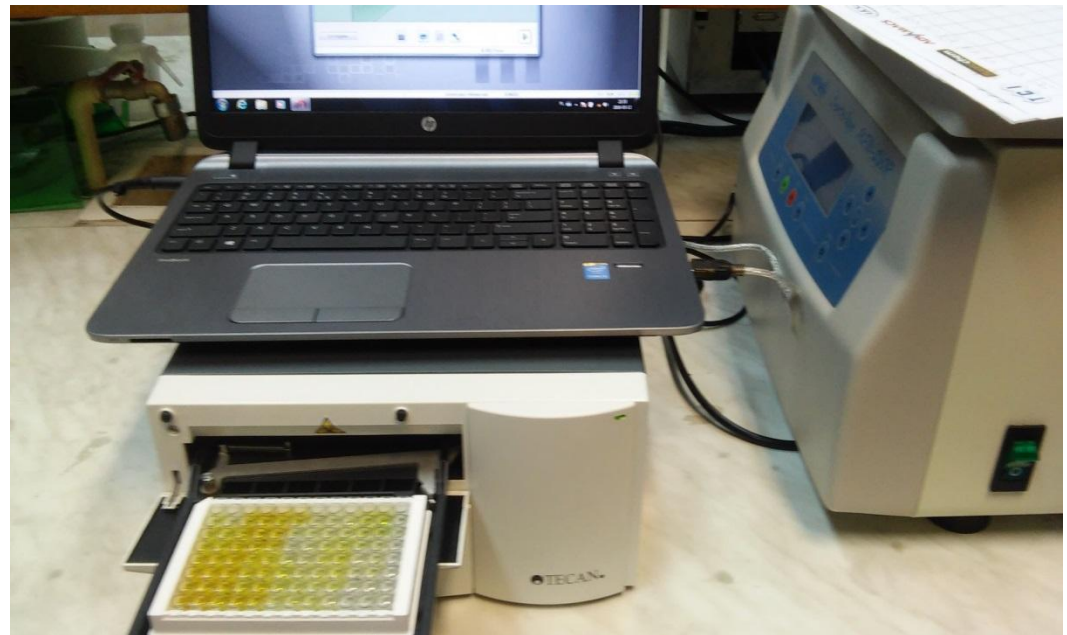
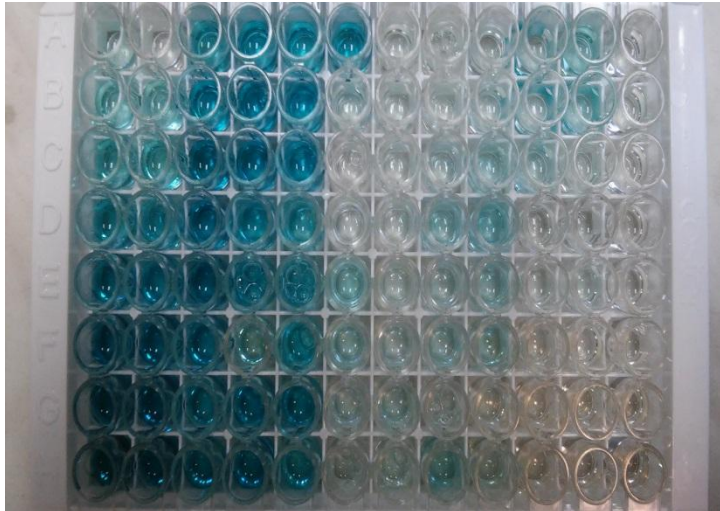
Blood analysis

Immunological parameters:

lysosyme activity,

Immunoglobulin IgA, IgM, IgY

proinflammatory cytokines - interleukin IL-6

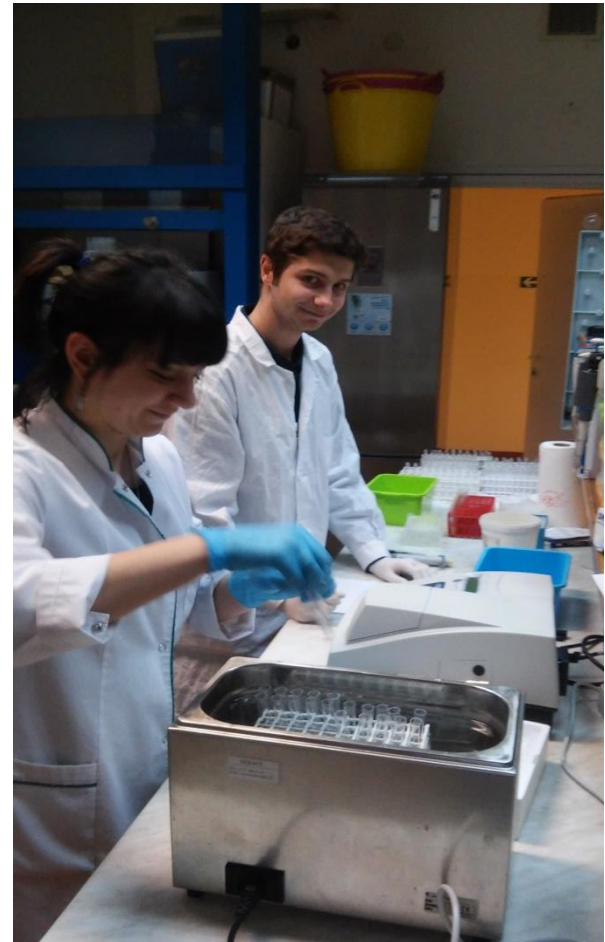


Parameters determined in biological material

Blood analysis

Biochemical parameters and redox status :

- total protein (TP),
- alanine aminotransferase (ALT),
- aspartate aminotransferase (AST),
- lactate dehydrogenase (LDH),
- urea (UREA),
- uric acid (UA),
- phosphorus (P)
- superoxide dismutase (SOD)
- catalase (CAT)
- Malondialdehyde (MDA)
- total antioxidants potential (FRAP)
- glutathione (GSSG+GSH)



Blood analysis



Results

The analyzed nutrient composition of **1 kg of mixture** for chicken

Group	STARTER (1-10 day of life)							
	EM MJ	Crude protein %	Crude fat %	Crude fibre %	Crude ash %	Starch %	Dry matter %	Humidity %
G-C	12.3	21.97	4.00	3.28	5.26	41.00	88.9	11.10
FS-3%	11.7	22.20	4.10	3.38	5.66	37.21	89.0	10.95
FS-6%	12.0	21.37	4.05	3.32	5.28	39.97	88.9	11.10
FR-3%	11.7	21.39	4.62	3.49	5.67	37.09	89.1	10.87
FR-6%	11.9	21.88	5.98	3.63	6.10	34.50	89.4	10.59

Results

The analyzed nutrient composition of **1 kg of mixture** for chicken

Group	GROVER 1 (10-23 day of life)							
	EM MJ	Crude protein %	Crude fat %	Crude fibre %	Crude ash %	Starch %	Dry matter %	Humidity %
G-C	12.4	20.89	5.28	3.24	4.99	40.27	88.4	11.60
FS-3%	12.3	22.29	4.69	3.19	5.11	39.50	88.3	11.71
FS-6%	12.2	22.25	4.48	3.22	5.10	39.46	88.6	11.34
FR-3%	12.4	20.97	5.97	3.37	5.23	38.54	88.5	11.51
FR-6%	12.5	21.00	7.00	3.61	5.65	36.83	88.5	11.42

Results

The analyzed nutrient composition of **1 kg of mixture** for chicken

Group	GROVER 2 (24-35 day of life)							
	EM MJ	Crude protein %	Crude fat %	Crude fibre %	Crude ash %	Starch %	Dry matter %	Humidity %
G-C	12.8	19.96	6.29	3.20	4.71	41.39	88.6	11.14
FS-3%	12.6	20.30	6.06	3.24	5.02	40.18	89.1	11.09
FS-6%	12.6	21.06	6.02	3.24	5.19	39.54	89.0	11.10
FR-3%	12.7	19.82	7.12	3.30	5.16	39.30	89.0	10.94
FR-6%	12.8	20.19	7.88	3.58	5.58	37.60	89.1	10.86

Results

The analyzed nutrient composition of **1 kg of mixture** for chicken

Group	FINISHER (35 – 42 day of life)							
	EM MJ	Crude protein %	Crude fat %	Crude fibre %	Crude ash %	Starch %	Dry matter %	Humidity %
G-C	12.5	20.92	4.99	3.19	4.80	41.15	88.6	11.39
FS-3%	12.6	19.27	5.53	3.21	4.88	42.04	88.4	11.53
FS-6%	11.3	19.78	6.99	3.37	5.05	39.79	88.8	11.14
FR-3%	12.6	20.31	6.18	3.28	5.10	40.27	88.6	11.33
FR-6%	12.7	19.56	7.14	3.46	5.34	39.55	88.7	11.27

Results

Content of phosphorus (P) and P-phytic of mixture feed

Group	Starter		Grower 1		Grower 2		Finisher	
	P (g kg ⁻¹)	P-phyt. (g kg ⁻¹)	P (g kg ⁻¹)	P-phyt. (g kg ⁻¹)	P (g kg ⁻¹)	P-phyt. (g kg ⁻¹)	P (g kg ⁻¹)	P-phyt. (g kg ⁻¹)
G-C	5.481	0.822	5.814	0.796	5.002	0.975	5.742	0.943
FS - 3%	5.424	0.752	5.696	0.883	5.115	0.919	5.854	0.850
FS - 6%	5.488	0.729	6.820	0.789	5.433	0.913	5.700	0.915
FR - 3%	5.569	0.859	7.140	0.791	5.585	0.928	5.103	0.924
FR - 6%	5.857	0.820	7.372	0.722	6.072	0.939	5.059	0.879

6% share of fermented soybean or rapeseed meal significantly increased the content of phosphorus in the Starter, Grower 1, Grower 2 and Finisher feeds.

In the samples of feed containing 6% fermented soybean or rapeseed meal a slight decrease in the content of phytate phosphorus was noted as well.

Results

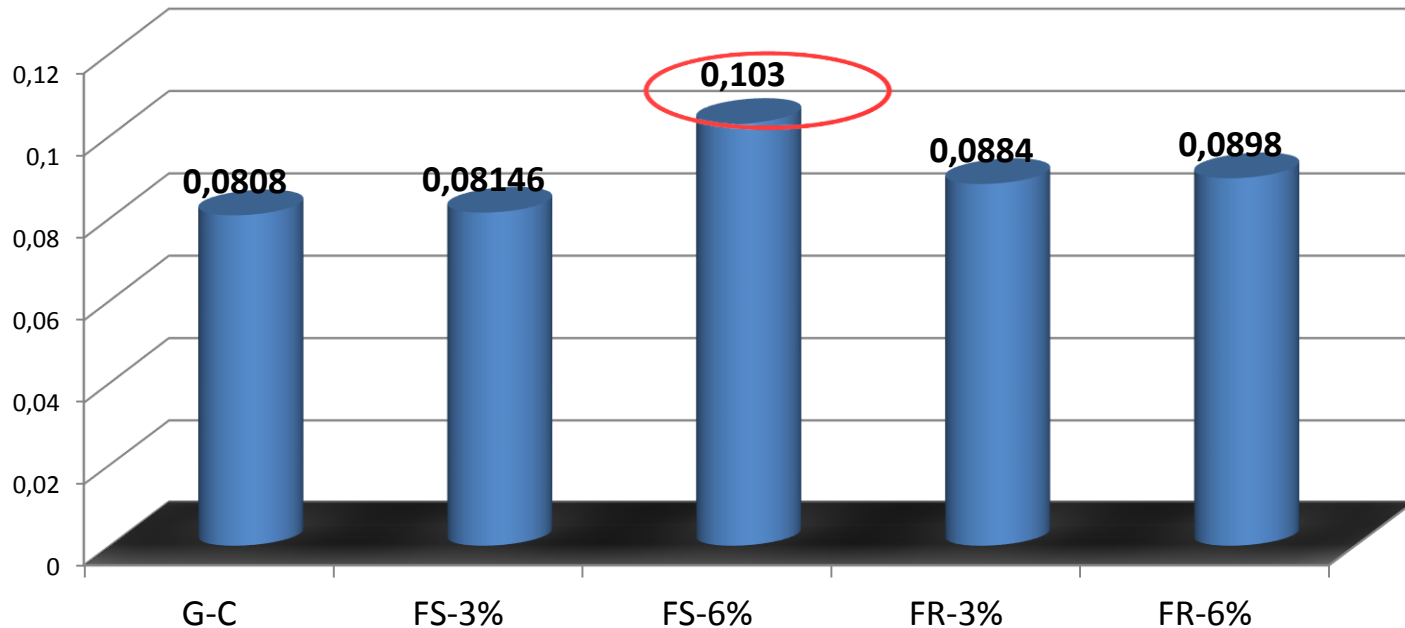
Performance effect of the chickens

Group	Body weight (kg)				FCR (kg kg ⁻¹)	Mortality (%)
	1st day	14th day	35th day	40th day		
Group						
G-C	0.042	0.370	1.782	2.106	1.693	2
FS – 3%	0.042	0.380 ^b	1.820 ^{ab*}	2.129 ^b	1.676 ^b	0
FS – 6%	0.042	0.413^{a*}	1.870^{a*}	2.156^{a*}	1.670^{b*}	0
FR – 3%	0.042	0.405 ^{ab*}	1.800 ^b	2.109 ^c	1.700 ^b	1
FR – 6%	0.042	0.382 ^b	1.820 ^{ab*}	2.100 ^c	1.758 ^{a*}	2
SEM	0.0001	0.001	0.016	0.015	0.012	-

Results

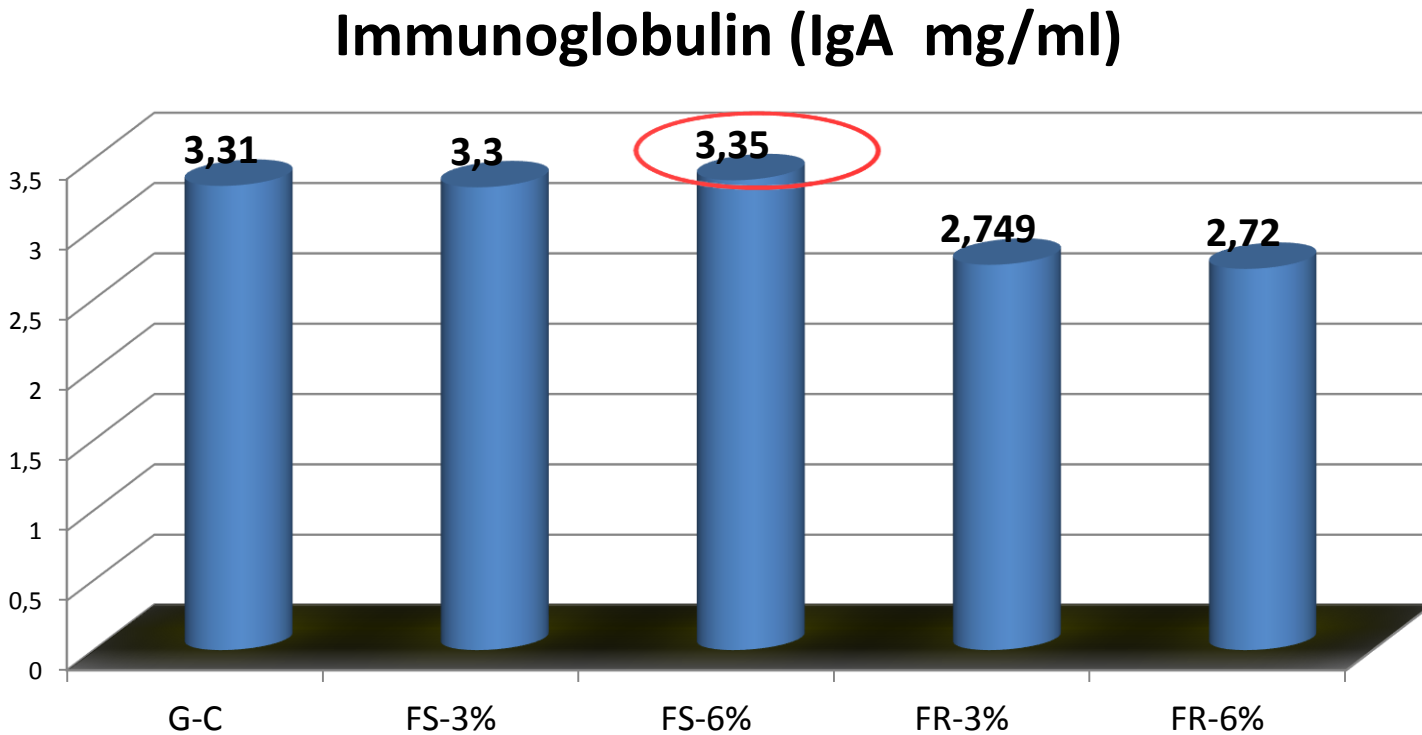
Immunological effect of the chickens

Interleucin (IL-6 pg/ml)



Results

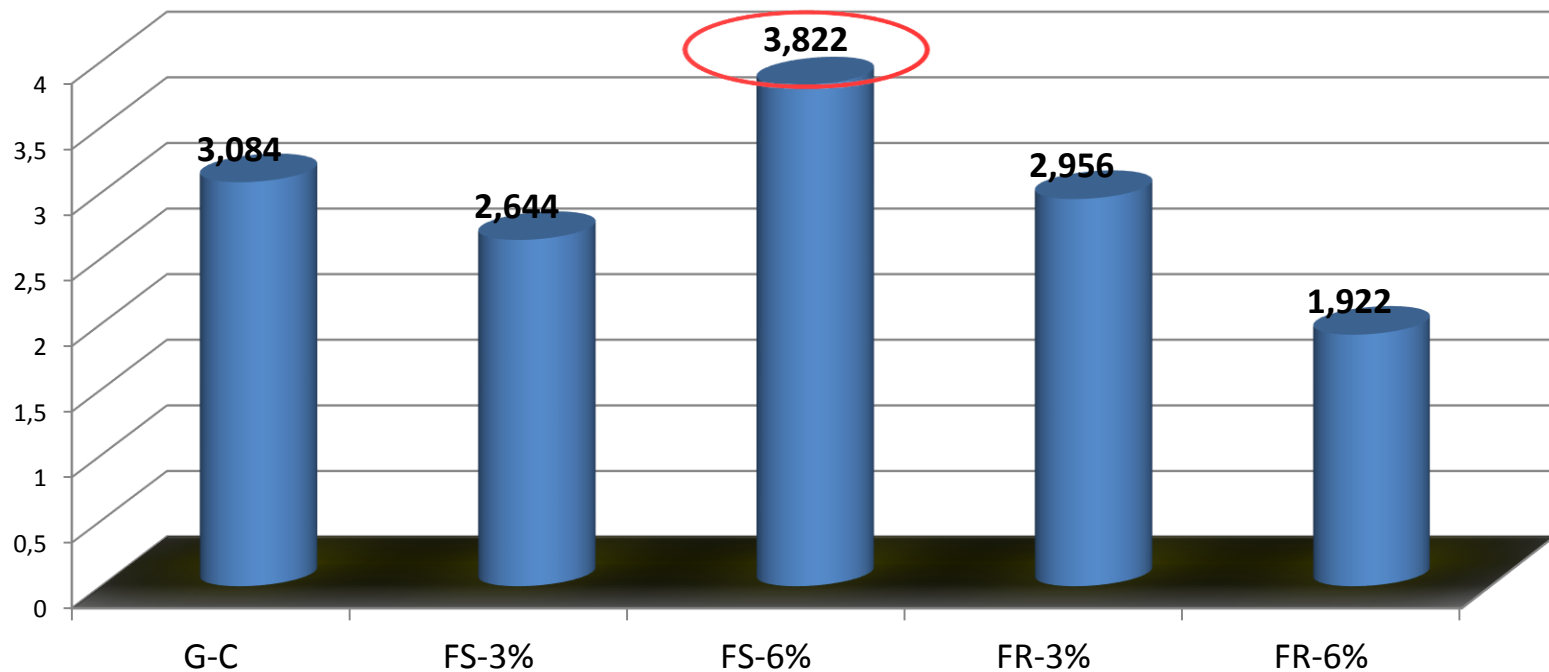
Immunological effect of the chickens



Results

Immunological effect of the chickens

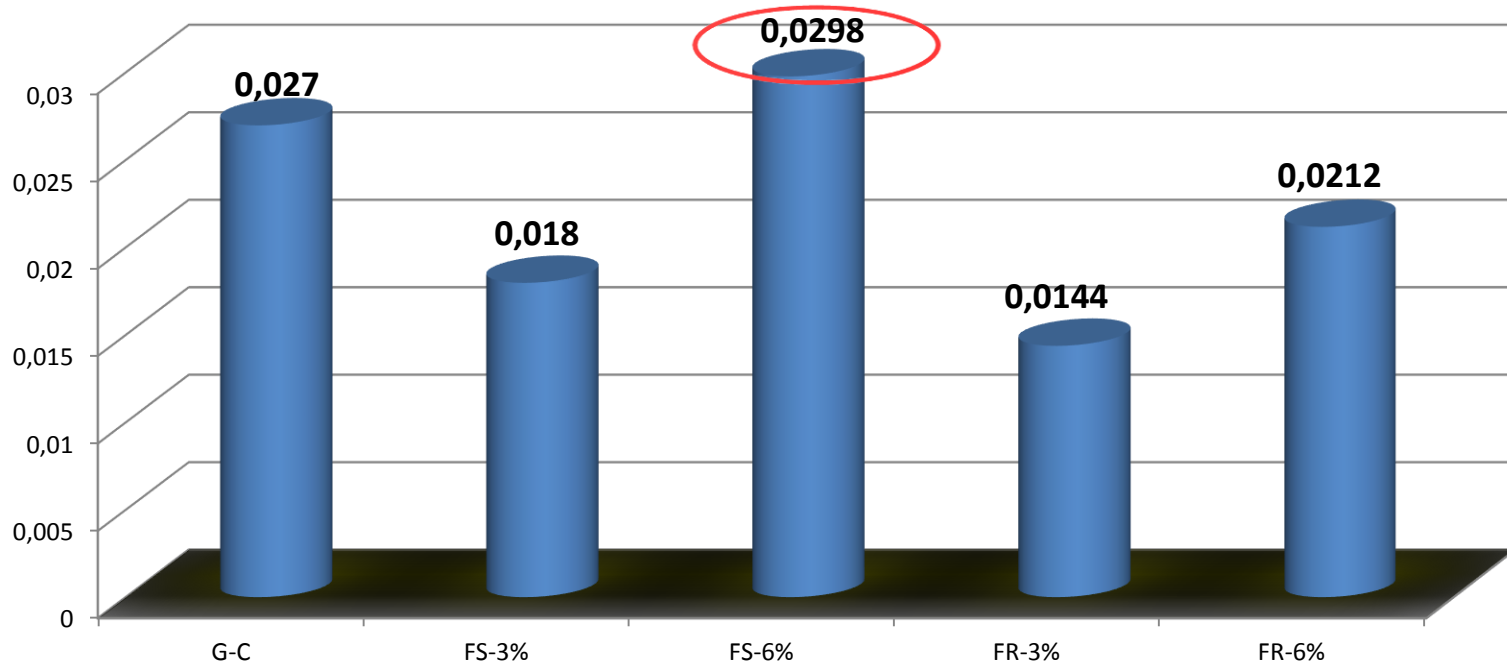
Lysozyme activity U/ml



Results

Immunological effect of the chickens

Ceruloplasmin activity U/ml



Conclusion

It has been found that an addition of **fermented soybean meal** in the amount of **6%** into the mixed feed gives the best effects on stimulating the answer of the immune system and antioxidative properties. The addition of this compound enables to obtain the **best productive efficiency**.

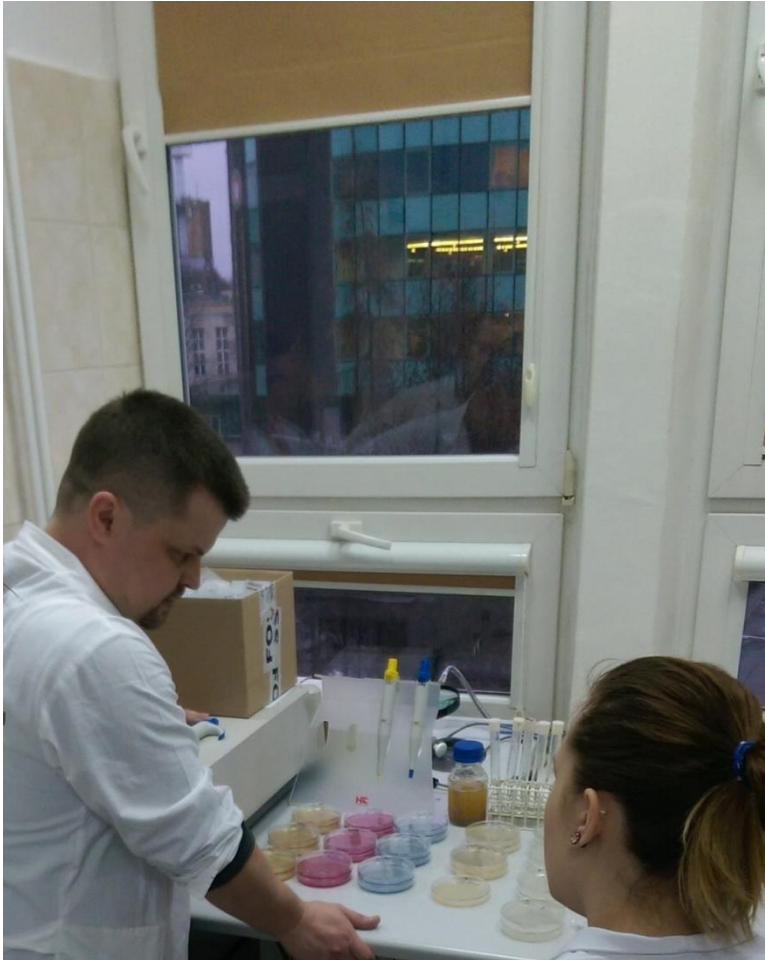
The microbiological analysis

- The content of intestines was collected into the sterile containers as a bulk sample from each of the analyzed groups.
- Then, weighted **20 grams of** collected contents were placed into the bottles containing 180 ml of the dilutions, and homogenized. The next step was to make a series of decimal dilutions and plate prepared samples for microbiological ground.

Parametres in the test

- The **total number of microorganisms** on nutrient agar (incubation at 37 degrees Celsius for 24 hours, followed by 3 days at 25 degrees Celsius and 3 days at 4 Degrees Celsius)
- The total number of **coliform bacteria** on agar endo les (incubation for 24 hours at 37 degrees Celsius)
- The total **number of *Escherichia coli*** on a substrate (FMC incubation for 24 hours at 37 degrees Celsius)
- The overall **number of fungi and mold's** on the ground Sabouarda of chloramfenicol (7 days incubation at 25 degrees Celsius)
- ***Campylobacter* species** on the ground **CCDA** (incubation for 48 hours in broth under microaerophilic then CCDA on an agar medium with antibiotics for 48 hours at 37 degrees Celsius)
- After an incubation period of grown bacterial colonies were counted and calculated in accordance with standards.

Seeding the samples for microbial substrate



The total number of microorganisms (cfu/g)

Experimental groups	GC	FS 3%	FS 6 %	FR 3%	FR 6%
Result (cfu/g)	184545	19091	19545	38636	65455
Result (log10)	5,2661033 53	4,28082 661	4,291045 775	4,586996 245	4,815939 811

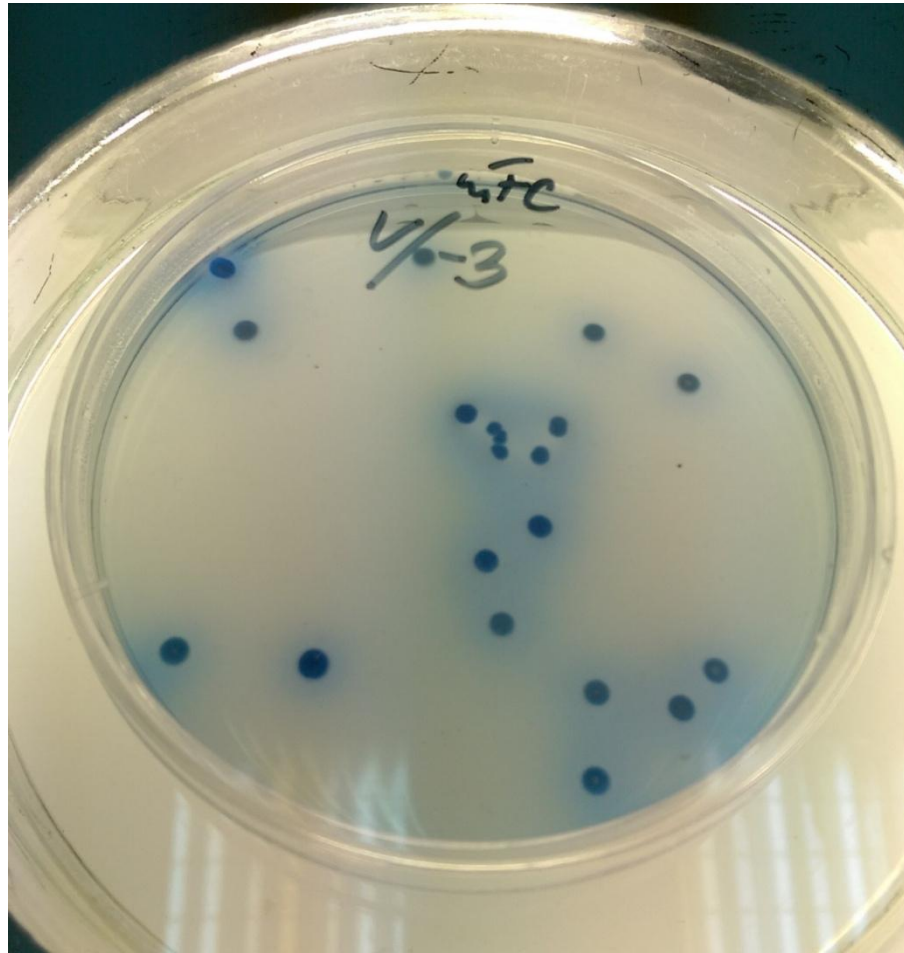
The overall number of fungi (cfu/g)

Experimental groups	GC	FS 3%	FS 6 %	FR 3%	FR 6%
Result (cfu/g):	45	23	14	5	0
Result (log10)	1,65	1,35	1,13	0,65	not present in 0,1g

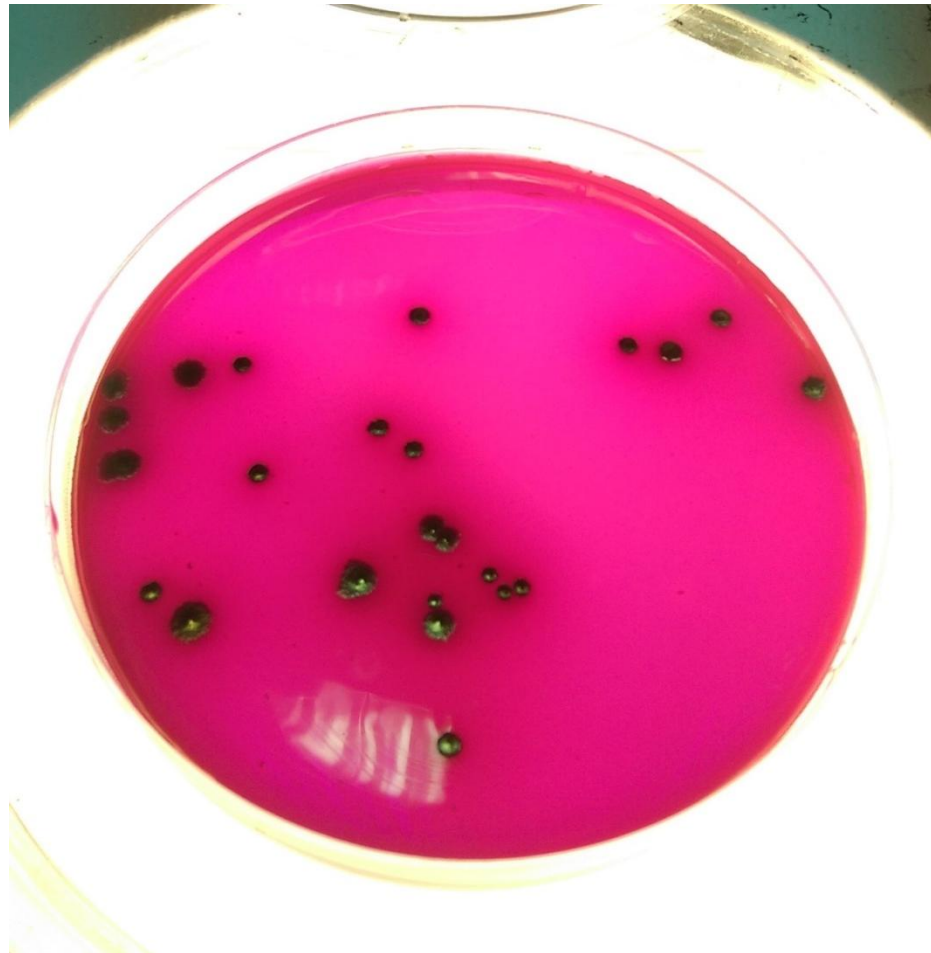
Total number of coliforms (cfu/g)

Experimental groups	GC	FS 3%	FS 6 %	FR 3%	FR 6%
Result (cfu/g):	3636	18182	909	455	35000
Result (log10)	3,5606	4,2596	2,9586	2,6575	4,5440

Escherichia coli on Mfc agar



Typical colonge of coliform bacterial on Endo Les agar



Result of histopathology examination

Measurements of the villi and crypts of the jejunum of the broiler chickens (μm)

Group	Mean length of villi of the jejunum (μm)	Mean depth of crypt of the jejunum (μm)
G-C	1499.5	188.5
FS-3%	1571.6	186.8
FS-6%	1785.9	212.9
FR-3%	1523.0	188.6
FR-6%	1572.1	185.8

The histological analysis of the jejunum of the chickens showed that the **6%** share of **fermented soybean** meal in the compound feed had a beneficial effect on the size of the villi and the depth of the crypts.

- During the production cycle of birds **cloaca swabs** were collected from each of the analyzed groups.
- In analyzed material after three times there were no *Salmonella* or *Campylobacter* in any of the analyzed groups.

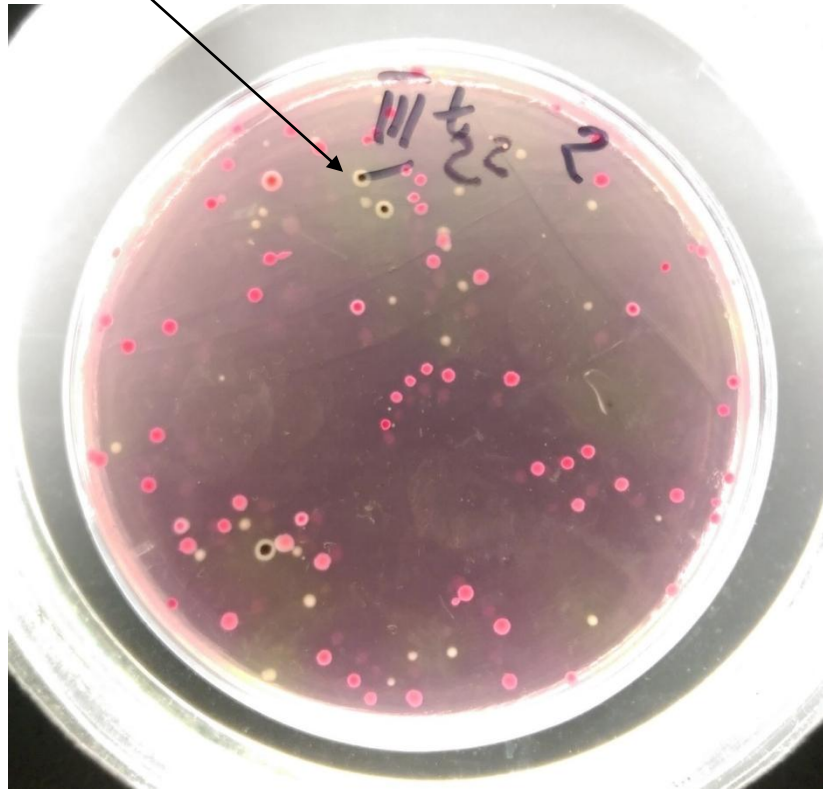
CDA

- The growth of microorganisms on the ground CDA taken as cloaca swabs of animals.

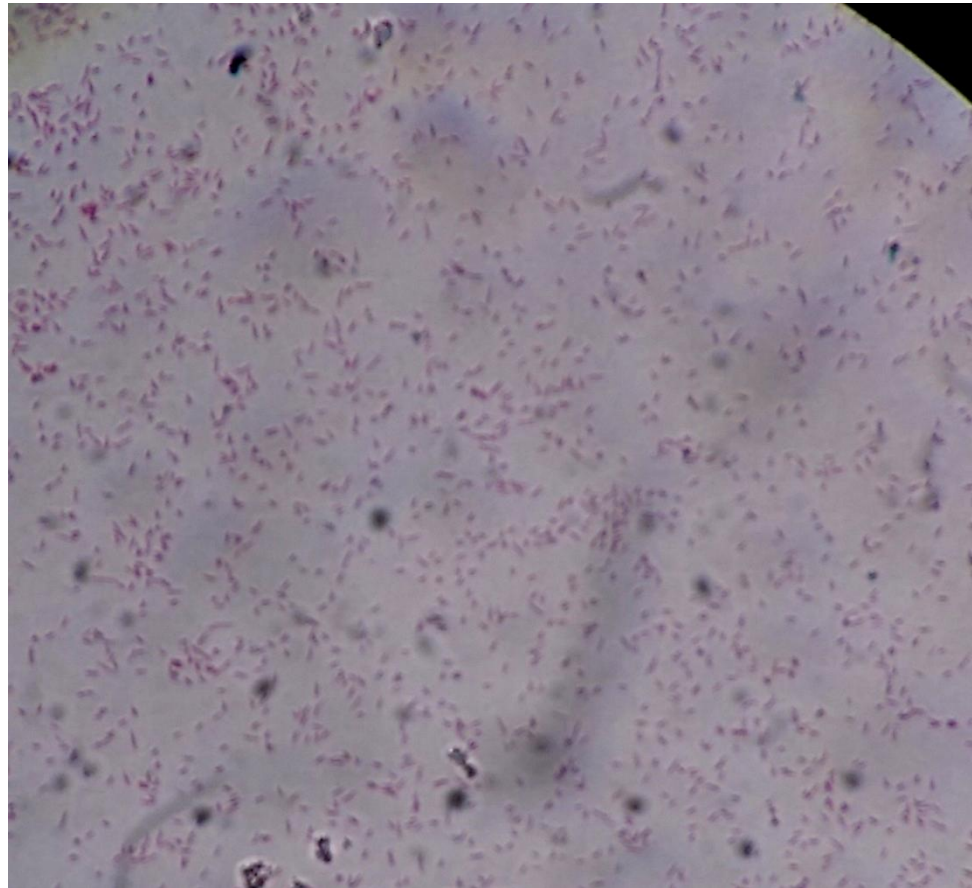


- Microorganisms that fulfill the criteria to be classified as *Salmonella* have been identified to species by isolating on the ground **Salmonella-Shigella** (SS agar) and with using of the appropriate **biochemical tests** (API analytical profile index) from the company BioMerieux.

Typical black colonies of *Salmonella* roads



The microscopic picture of gram-negative bacilli



API test





API 20 E V5.0

[Drukuj](#)

[Wyślij](#)

[Nowy test](#)

[Modyfikuj](#)

WYNIK

DATA

KOMENTARZ

API

- API 10S
- API 20 A
- API 20 C AUX
- API 20 E
- API 20 NE
- API 20 STREP
- API 50 CHB
- API 50 CHE
- API 50 CHL
- API CAMPY
- API CANDIDA
- API CORYNE
- API LISTERIA
- API NH
- API STAPH
- RAPID 20 E

BARDZO DOBRA IDENTYFIKACJA

Pasek	API 20 E V5.0
Profil	0 7 3 7 0 0 0
Uwaga	

Takson istotny	% ID	T	Testy przeciwne			
Proteus mirabilis	99.9	0.67	VP	1%		

Następny takson	% ID	T	Testy przeciwne							
Proteus vulgaris group	0.1	0.0	ODC	1%	CIT	12%	IND	92%	VP	1%
			SAC	89%						

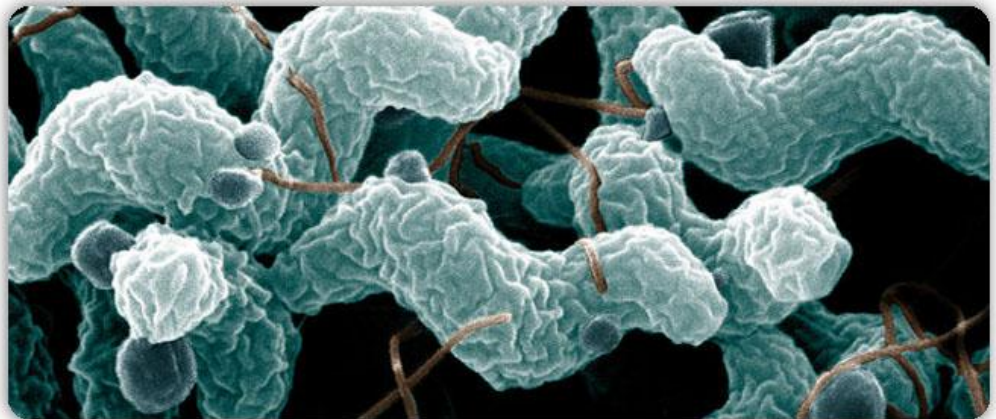
ID32

A thorough analysis of selected colonies ruled out the presence of *Salmonella* pointing to *Proteus mirabilis*

The presence of *Salmonella* and *Campylobacter*

Experimental groups	GC	FS 3%	FS 6 %	FR 3%	FR 6%
Salmonella sp.	Not present in 0,1g				
Campylobacter sp.	Not present in 0,1g				

- Also in the analyzed content of **colorectal** chickens there was no presence of the bacteria that fulfill the criteria for *Campylobacter* species.



Conclusion

- It has been found that use of fermented protein products (which contain lactic acid) in mixed feed for chicken can **limit** the number of **pathogenic microorganisms**. Furthermore it will protect the feed from developing these species of fungi, which are capable to product toxins. Beneficial reduction of the molds' number can be a natural method to **fortify** the feed from adverse **enzymatic changes** (fungal enzymes) and the cumulation of **mycotoxins** in the feed.

Result

Content of P, Ca and Mg in the blood plasma of the chicken

Group	P (mmol l ⁻¹)	Ca (mmol l ⁻¹)	Mg (mmol l ⁻¹)
Group			
G-C	1.725	1.788	0.352
FS - 3%	1.708	1.871	0.337
FS - 6%	1.620	2.238	0.371
FR - 3%	1.702	2.018	0.334
FR - 6%	1.795	2.132	0.385

Conclusion

- By introducing fermented soybean or rapeseed meal to chicken feed in the amount of 6% it is possible to increase the availability of **phosphorus** from compound feed and improve the degree of **calcium** bioretention in chickens.

Result

Slaughter analysis of the chickens

Group	Dressing Percentage %	Breast Muscles %	Thigh Muscles %	Drumstick %	Liver %	Stomach %	Heart %	Abdominal Fat %	Thighbone %	Shinbone %
Group G-C	74.78	25.82	10.04	7.39	2.82	1.43	0.57	0.59	1.06	1.81
FS - 3%	81.10	27.07	10.51	8.18	2.59	1.40	0.48	0.49	0.97	1.74
FS - 6%	83.90	26.37	9.86	7.30	2.67	1.32	0.45	0.32	0.72	1.81
FR - 3%	79.71	26.85	8.84	7.77	2.28	1.46	0.52	0.57	0.95	1.84
FR - 6%	85.33	26.16	8.87	6.82	2.70	1.37	0.52	0.62	0.79	1.93

Result

Haematological parameters in the blood of the chickens

Group	WBC (10^9 l^{-1})	LIMF %	HETERO %	RBC (10^{12} l^{-1})	Hb (g l^{-1})	Ht (l l^{-1})
Group						
G-C	25.88	71.62	27.80	2.216	15.82	25.09
FS - 3%	22.33	73.14	26.32	2.448	16.02	25.94
FS - 6%	24.94	70.20	29.22	2.256	15.42	24.65
FR - 3%	26.21	55.20	44.22	2.090	14.82	23.43
FR - 6%	24.41	63.90	33.70	2.252	15.06	24.92

The lowest **WBC** count was noted in group FS3%. In the groups receiving fermented rapeseed meal (FR3% and FR6%) a reduced percentage of **lymphocytes** was noted, accompanied by an increase in the percentage of **heterophils**.

Result

Redox parameters in the blood of the chickens

Group	SOD (U ml ⁻¹)	CAT (U ml ⁻¹)	Cp (U l ⁻¹)	Zn (μmol l ⁻¹)	Cu (μmol l ⁻¹)	Fe (μmol l ⁻¹)	IBC (μmol l ⁻¹)
Group							
G-C	25.15	6.896	0.027	71.21	2.279	37.50	21.00
FS - 3%	25.08	5.793	0.018	98.21	2.886	35.75	29.25
FS - 6%	25.09	7.122	0.029	107.5	3.129	21.50	26.25
FR - 3%	25.10	6.620	0.014	80.55	3.849	37.75	25.50
FR - 6%	25.07	7.172	0.027	158.1	3.793	19.27	33.25

Result

Redox parameters in the blood of the chickens

Group	LOOH ($\mu\text{mol l}^{-1}$)	MDA ($\mu\text{mol l}^{-1}$)	FRAP ($\mu\text{mol l}^{-1}$)	GSSG+GSH ($\mu\text{mol l}^{-1}$)
Group				
G-C	20.10	1.158	103.3	0.188
FS - 3%	17.00	1.104	131.9	0.207
FS - 6%	17.64	1.194	139.7	0.208
FR - 3%	22.28	1.270	93.75	0.263
FR - 6%	24.05	1.217	95.48	0.265

The fermented rapeseed meal component (FR3% and FR6%) in the compound feed caused a detrimental increase in the concentration of **MDA** in the blood plasma of the chickens. The 6% share of fermented soybean meal beneficially increased **FRAP** in the blood plasma of the chickens.

Result

Activity enzyme in the blood of the chickens

Group	AST (U l ⁻¹)	ALT (U l ⁻¹)	CK (U l ⁻¹)	GGT (U l ⁻¹)
Group				
G-C	224.1	4.896	2720.0	30.12
FS - 3%	264.9	2.174	3227.2	36.09
FS - 6%	295.0	4.365	2828.7	26.14
FR - 3%	202.5	4.990	3723.3	44.05
FR - 6%	219.4	3.534	3288.6	44.03

Result

Biochemical parameters in the blood of the chickens

Group	TP (g l ⁻¹)	ALB (g l ⁻¹)	UA (mmol l ⁻¹)	UREA (mmol l ⁻¹)	BIL (μmol l ⁻¹)
Group					
G-C	28.29	0.010	141.2	1.366	18.06
FS - 3%	32.73	0.011	146.6	1.010	12.64
FS - 6%	31.35	0.010	142.6	1.090	9.030
FR - 3%	29.91	0.010	150.5	1.544	11.74
FR - 6%	29.18	0.009	196.1	2.495	12.94

Result

Biochemical parameters in the blood of the chickens

Group	GLU (mmol l ⁻¹)	CREAT (μmol l ⁻¹)	CHOL (mmol l ⁻¹)	HDL (mmol l ⁻¹)	TG (mmol l ⁻¹)
Group					
G-C	13.67	21.58	3.054	1.695	0.570
FS - 3%	13.29	25.04	3.297	1.832	0.554
FS - 6%	12.76	15.54	2.969	1.813	0.510
FR - 3%	13.03	22.44	2.954	1.655	0.642
FR - 6%	13.90	24.17	3.554	2.201	0.615

Conclusion

- The introduction of fermented soybean meal to compound feed in the amount of 6% results in the most effective stimulation of the antioxidant response and to the best growth performance.

IMPORTANT

- During experiment, chickens (control group) which did not receiving fermented product had diarrhoea (a symptom of wet hen house). In the experimental groups receiving fermented product (FS-3% or 6%, FR - 3% or 6%) bedding was dry by all experiment.
- On reduce the problem of wet bedding was influenced: decrease the number of bacteria and fungi in the intestine, a beneficial effect on intestinal histology, improving immunity of chickens.