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The use of chosen plant extracts in various aspects of Japanese quail production

Summary of doctoral dissertation accomplishments

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Introduction

Plants and their extracts have been used since ancient times. They are valued because of their specific aroma and various curative properties. Most of the plants contain antymicrobial compounds for the prophylactic and therapeutic activity against bacteria, fungi, protozoa, yeasts and molds. Active molecules of plants with anti-inflammatory activity include terpenes, carotenoids, phenolic substances and flavonoids. Substances included in these plants increase the activity of lymphocytes, macrophages, and natural killer (NK) cells. They improve phagocytosis or/and stimulate the synthesis of interferon.

Modern science has proven that plants and herbal materials, containing bioactive compounds, may be applied not only in human medicine, but can also significantly improve production, physiological and reproductive effects of animals, and have a therapeutic effect against many diseases that affect them. Herbs and spices began to be used in animal nutrition in order to increase the efficiency of feed nutrients utilization. Their active ingredients improve digestion and stimulate cell metabolism.

Herbs and their extracts or essential oils contain bioactive compounds, which may improve the quality of poultry products (meat and eggs) and their use as antioxidants is not only important to the health of the birds, but also for the oxidative stability of raw materials derived from them. Plants are worth attention, as may contribute to a significant improvement in production effects, health status and thus welfare of poultry, and provide a safe alternative to feed antibiotics.

The aim of study and research hypotheses

The aim of the study was to evaluate the possibility of using selected natural plant extracts, ginger (GR, *Zingiber officinale*), garlic (GC, *Allium sativum*), oregano (O, *Origanum vulgare*) and cinnamon (C, *Cinnamomum zylenicum*) in various aspects of poultry production based on the example of Japanese quail.

Based on the available references, three research hypotheses were formulated :

1. Natural ingredients contained in the plant material may have an inhibitory effect on the growth of microorganisms during the incubation of hatching eggs;
2. *In ovo* injection of plant extracts solutions during incubation may affect hatching results of birds, stimulate the embryonic development and later modify parameters associated with productive and physiological performance;

3. Addition of plant extracts to feed may improve production indicators related to the eggs obtaining and alleviate the oxidative stress in birds.

Because of the broad scope of issues, in order to verify research hypotheses three separate experiments were conducted, materials and methods of each experiment were described for each of them specifically.

The research was conducted with the approval of the 2nd Local Ethical Committee for Animal Experiments in Lublin (Permission No. 16/2014 of 17/11/2017).

Experiment I

The aim of study was to evaluate of antimicrobial properties for aqueous extracts of ginger (GR), garlic (GC), oregano (O) and cinnamon (C) as safe and alternative substances for traditional disinfectant of hatching eggs which were represented by formaldehyde gas and may exhibit adverse effects of avian embryos and human health through disinfection process of hatching eggs in hatcheries and influence this procedure in hatchability results as well as resulted chicks quality.

Materials and methods

The materials consisted of 2400 hatching eggs (weight about 10.5 g) obtained from Japanese quails layers at the age of 14 week. Eggs were divided randomly into 6 groups before incubation, 400 eggs per group (4 replication subgroups in each). Eggs from 1st group were not disinfected (NC, negative control), eggs from 2nd group were disinfected by fumigation with formaldehyde gas (PC, positive control). For disinfection in groups 3rd, 4th, 5th and 6th , aqueous extracts (5%) of GR, GC, O, and C were used respectively. Eggs were hatched artificially using a BIOS hatching apparatus under standard conditions of incubation:

- setting compartment - 37.6–38.0°C temp. and 50–65% relative humidity.
- hatching compartment - 37.0–37.5°C temp. and 75–80% relative humidity.

After hatch, all birds were kept under uniform management conditions up to 14 days of rearing. The birds were fed *ad libitum* by balanced mixture dedicated to meat-type quails.

Studied characteristics

All eggs including fertile, infertile and unhatched eggs were weighed with a digital scale and eggshell conductance constant (*K*) were determined as criterion of egg weight loss. Hatched chicks, dead embryos and crippled chicks were calculated. Also, body weight and survivability of chicks were registered on 1st, 7th and 14th day of their lives.

Egg samples for microbial screening were collected on 14th day of experiment. 12 eggs per each group were placed in sterile boxes containing 50 ml of phosphate-buffered saline (PBS) with 3 drops of TWEEN 80. The samples were serially diluted in PBS and plated on sterile medium in order to obtain the total number of bacteria, yeast and fungi. After incubation colonies were counted and presented as cfu/1ml of liquid from the egg. To identify the bacterial colonies, a microscopic examination was performed as well as Gram's staining method and API biochemical tests.

The data were analyzed with the use of statistical package SPSS 20.0PL. The normality of data was verified using Kolmogorov-Smirnov test. The significance level was defined as 5% ($p \leq 0.05$). The one-way ANOVA with Tukey's test was carried out. The mortality, hatchability and bacterial number of colony forming units were verified using non-parametrical χ^2 test.

Results

Depending on group, table1 shows that lack of differences was obvious in hatchability, total mortality on fertile eggs and crippled chicks. All disinfectant groups and NC did not significantly differ in eggshell conductance const (*K*). In weight loss of fertile eggs, disinfectant solution of C led to reduction of weight loss of fertile eggs (%).

Table 1. Hatchability traits of Japanese quails influenced by eggs disinfection with aqueous solutions of plant extracts

Traits	Groups						χ^2 *(p-value)*
	NC	PC	GR	GC	O	C	
Hatchability of fertile eggs (%)	81.20	84.85	71.97	73.23	83.09	80.80	*0.940*
Total mortality of fertile eggs (%)	18.80	15.15	28.03	26.77	16.91	19.20	*0.242*
Crippled chicks (%)	0.00	0.00	0.00	0.00	0.00	0.00	-
							SEM
Eggshell conductance constant (*K*) of fertile eggs	0.151^{ab}	0.208^{a}	0.182^{ab}	0.207^{ab}	0.165^{ab}	0.111^{b}	*0.009*
Weight loss (%) of fertile eggs	10.01^{a}	14.07^{a}	12.29^{a}	28.86^{a}	11.16^{a}	7.51^{b}	*1.467*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon;$^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

No differences between GR, GC, O were stated as well as between NC and PC in body weight at hatch and at 14th day. Lack of differences in survivability in post–hatch depending on group was recorded (table 2).

Table 2. Body weight and survivability of Japanese quails influenced by eggs disinfection with aqueous solutions of plant extracts

Traits	Groups						SEM
	NC	PC	GR	GC	O	C	
Body weight at							
hatch	6.93^{a}	6.80^{ab}	6.89^{a}	6.66^{ab}	6.78^{ab}	6.28^{b}	*0.061*
7th d	19.82^{ab}	19.20^{ab}	20.70^{a}	18.31^{b}	15.88^{c}	14.53^{c}	*0.290*
14th d	42.35^{ab}	42.96^{ab}	44.06^{ab}	44.54^{a}	39.92^{b}	32.27^{c}	*0.565*
							χ^2 *(p-value)*
Total survivability (%)	96.84	97.62	98.95	100.00	100.00	96.84	*0.190*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

Table 3. Microbial counts on egg shell of Japanese quails influenced by eggs disinfection with aqueous solutions of plant extracts

Traits	Groups						
	NC	PC	GR	GC	O	C	SEM
Total number of fungi*	0.96^{b}	1.36^{a}	1.18^{a}	0.00	1.00^{b}	0.92^{b}	*0.285*
Total number of bacteria*	1.67^{c}	1.53^{c}	1.70^{c}	2.17^{b}	2.55^{a}	2.14^{b}	*0.062*
Identified bacteria species**							χ^2 *(p-value)*
E. coli	9.80	14.6	10.6	0.00	0.80	8.10	*0.025*
Salmonella spp.	7.60	8.30	23.4	0.00	0.00	0.00	*0.000*
Staphylococcus aciuri	44.6	0.00	0.00	0.00	0.00	0.00	*0.000*
Staphylococcus epidermidis	13.0	0.00	0.00	40.0	0.00	5.40	*0.000*
Staphylococcus spp.	5.40	20.8	31.9	0.00	0.00	14.9	*0.000*
Streptococcus spp.	17.4	50.0	14.9	60.0	99.2	60.8	*0.000*
Non identified bacteria	2.20	6.30	19.1	0.00	0.00	10.8	*0.002*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for groups) differ significantly at $p \leq 0.05$; * – Log_{10} CFU/1ml; ** – % of total isolates

Table 3 revealed the microbial counts of egg shell under disinfectants effect. In GC, no fungal colonies were found. In terms of identified bacteria species, *E. coli* in GC, *Salmonella* spp. in (GC, O, C), *Staphylococcus aciuri* in (PC, GR, GC, O, C), *Staphylococcus epidermidis* in (PC, GR, O), *Staphylococcus* spp. and non-identified bacteria in (GC and O) have not been detected.

Experiment II

The aim of the study was to evaluate the potential influence for aqueous extracts of ginger (GR), garlic (GC), oregano (O) and cinammon (C) as natural substances injected *in ovo* to Japanese quail hatching eggs on hatchability performance, productive and physiological status of hatched chicks. The sexual maturity characteristics of females was also monitored. It was hypothesized that plant extracts can perform two positive effects of avian embryos: as natural growth promoters and as an antioxidant.

Materials and methods

The materials consisted of 2400 hatching eggs. All eggs were numbered individually and allocated randomly into 6 groups before incubation, 400 eggs per group (4 replicates in each). On 5^{th} day of incubation, eggs from each treatment were removed from the setter to perform *in ovo* injection by 1% solution of plant extracts (0.1 ml) into air cell. The same managment procedures of hatching conditions to incubate eggs and rearing the chicks in post hatch were used as in the previous experiment up to 7 wks.

Studied characteristics

The same traits to evaluate hatching results were calculated as in previous experiment. After hatch, weekly body weight, feed intake, feed conversion ratio, protein efficiency ratio, energy efficiency ratio, production efficiency factor and mortality were recorded. After slaughter the simplified dissection of carcasses was done. Proportions of particular elements in carcass as well as proportions of giblets and abdominal fat in body weight were determined.

The meat pH value (15 and 60 minutes, as well as 24 hours after slaughter), thermal loss and water-holding capacity of meat were also determined.

Gastrointestinal tract morphometry determined using a measuring tape and vernier caliper. 1 g sample from digesta content of each gastrointestinal segment was taking to evaluate its pH.

The duodenal segments were placed in plastic tubes and fixed in 4% buffered formalin solution, dehydrated, and embedded in paraffin wax. The 4 μm – thick sections were stained with (H&E) for processing. The measurements for the villus and crypt dimensions were implemented using optical microscope equipped with camera and computer with measurement scale and imaging software.

Blood samples from birds were collected from the brachial vein and placed in tubes containing K3-EDTA. In the full blood: red blood cells (RBC) number, white blood cells (WBC) number, haemoglobin (Hgb) content and packed cell volume (PCV) were determined manually.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also calculated. The antioxidative enzymes concentrations including superoxide dismutase (SOD) and catalase (CAT) were calculated and levels of redox status indicators including lipid hydroperoxide (LOOH), malondialdehyde (MDA) were calculated as well. Glucose (GLU), total protein (TP), creatinine (CREAT), triglycerides (TG), total cholesterol (CHOL), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL). The following enzymatic indicators were also analyzed: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP) and levels of calcium (Ca), and phosphorus (P) determined spectrophotometrically. For the determination of nonesterified fatty acid (NEFA) concentrations by using a colorimetric method.

The age of sexual maturity of females was monitored and detected by laying of 1st egg. Daily enumeration of total produced eggs till 7 wks were done.

The data were analysed with the use of the statistical package SPSS 20.0PL. The Kolmogorov–Smirnov test was carried out in case of normality of data. The significance level was defined as 5%. The obtained numerical data were verified by t-test and one- or two-factorial ANOVA and Tukey's test. The statistical model incorporated the *in ovo* injection (IO) groups and the interaction between IO and sex were compiled as well. The χ^2 test was used to analyse non-parametric data of experiment (i.e. mortality and hatchability).

Results

The obtained findings in table 4 confirmed that was all plant extracts groups lead to reduce all hatching results. NC had the best hatching results in relation to other groups. The GR group had the lowest values in *K* and weight loss of fertile eggs.

In table 5 production results of birds are presented. Hatchlings in C group had the biggest body weight on 1st day than in other groups. In final body weight, all *in ovo* injection groups registered a numerical increase in relation to NC group. With respect to feed intake, all *in ovo* injection groups showed higher level of this parameter. However, in case of feed conversion ratio, protein and energy efficiency ratios, its total values were similar regardless of the group. All *in ovo* injection groups recorded significantly lower total mortality in total, compared to NC.

Table 4. Hatchability traits of Japanese quails influenced by *in ovo* injection with aqueous solutions of plant extracts

Traits	Groups						χ^2 *(p-value)*
	NC	PC	GR	GC	O	C	
Hatchability of fertile eggs (%)	88.20	83.80	65.50	58.40	44.00	59.70	*0.000*
Total mortality of fertile eggs (%)	11.82	16.18	34.47	41.59	55.46	40.30	*0.000*
Crippled chicks (%)	0.000	1.150	0.560	4.700	0.560	1.420	*0.000*
							SEM
Eggshell conductance constant (*K*) of fertile eggs	0.179[a]	0.173[a]	0.135[b]	0.169[a]	0.180[a]	0.182[a]	*0.003*
Weight loss (%) of fertile eggs	12.95[a]	12.56[a]	9.79[b]	12.21[a]	13.04[a]	13.20[a]	*0.248*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; [a, b] - means within rows (for groups) differ significantly depending on test at $p \leq 0.05$

Table 5. Total productive performance up to 7 [th] wk influenced by *in ovo* injection with aqueous solutions of plant extracts

Traits	Groups					SEM
	NC	GR	GC	O	C	
Body weight at hatch (g)	6.18[b]	6.18[b]	6.10[b]	6.11[b]	6.41[a]	*0.038*
Final body weight (g)	162.72	170.89	172.84	174.09	169.75	*1.391*
Feed intake (g)	689.83[b]	737.63[a]	759.95[a]	760.35[a]	723.43[a]	*9.295*
Feed conversion ratio (g/g bw)	4.42	4.51	4.57	4.54	4.43	*0.059*
Protein efficiency ratio (g/g/d)	1.001	0.984	0.966	0.973	1.002	*0.015*
Energy efficiency ratio (g/100 kcal/d)	8.42	8.27	8.12	8.18	8.42	*0.123*
Production efficiency factor (pts.)	6.78	7.45	7.36	7.62	7.68	0.182
Mortality (%)	10.73[a]	4.60[b]	4.95[b]	2.88[c]	2.63[c]	*1.230*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; [a, b] – means within rows (for groups) differ significantly at $p \leq 0.05$

Carcass yield in table 6 was influenced by the injected factor, considerably the biggest proportion of carcass in body weight was stated in GR group. A decrease in abdominal fat pad proportion was observed as a result of injection *in ovo* in GR, GC and C groups also, ginger increased liver and gizzard shares.

Table 6. Final carcass yield and carcass cuts at 7th wk influenced by *in ovo* injection with aqueous solutions of plant extracts

Traits	Groups					SEM
	NC	GR	GC	O	C	
Carcass yield (%)	64.04^{ab}	66.21^{a}	63.86^{b}	62.22^{b}	64.47^{ab}	*0.508*
Breast (%)	29.37	27.55	28.28	29.76	27.98	*0.288*
Thighs (%)	23.35	23.11	23.17	23.19	23.42	*0.179*
Drumsticks (%)	8.04^{a}	9.91^{a}	6.13^{b}	6.22^{b}	6.38^{b}	*0.275*
Wings (%)	9.99	9.29	9.89	8.33	9.71	*0.165*
Trunk (%)	29.25	30.14	32.53	32.50	32.51	*0.468*
Abdominal fat (%)	1.929^{a}	0.965^{b}	0.723^{b}	1.215^{ab}	1.042^{b}	*0.084*
Heart (%)	0.896	0.978	0.993	0.941	0.931	*0.015*
Liver (%)	2.104^{b}	2.454^{a}	2.256^{b}	2.210^{b}	2.388^{ab}	*0.045*
Gizzard (%)	1.720^{b}	2.118^{a}	1.872^{ab}	1.767^{b}	1.729^{b}	*0.031*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

Table 7. Meat quality at 7th wk influenced by *in ovo* injection with aqueous solutions of plant extracts

Traits	Groups					SEM
	NC	GR	GC	O	C	
Breast muscle						
pH_1 (15 min.)	5.89^{ab}	5.81^{b}	5.86^{b}	6.11^{a}	5.91^{ab}	*0.023*
pH_2 (1 hr)	5.88	5.89	5.90	5.83	5.85	*0.014*
pH_3 (24 hrs)	5.92	5.85	5.88	5.89	5.93	*0.015*
Thermal loss (%)	27.42	23.55	24.35	25.73	22.50	*0.925*
WHC (cm^2)	9.18	8.60	8.65	7.87	8.65	*0.404*
Thigh muscle						
pH_1 (15 min.)	6.39^{b}	6.44^{ab}	6.55^{ab}	6.63^{a}	6.32^{b}	*0.025*
pH_2 (1 hr)	6.41	6.44	6.37	6.37	6.41	*0.022*
pH_3 (24 hrs)	6.56^{ab}	6.58^{ab}	6.65^{a}	6.47^{b}	6.58^{ab}	*0.017*
Thermal loss (%)	28.79	25.58	27.80	25.19	24.85	*1.123*
WHC (cm^2)	7.18	8.01	9.02	7.62	7.70	*0.520*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

Generally, differentiation of traits did not result from injected preparations. However, in O group, pH_1 of thigh muscle measured 15 minutes after slaughter was significantly the biggest, in relation to other groups (table 7).

In table 8 anatomical traits and pH of gastrointestinal tract content are showed. The gastrointestinal tract of birds from injected groups were heavier and longer. *In ovo* injection

increased most of morphometrical parameters. In case of pH in gizzard GC, O and C decreased its value significantly. Also, large intestine pH of C was lower than others.

Table 8. Gastrointestinal tract morphology and its acidity in Japanese quails influenced by *in ovo* injection with aqueous solutions of plant extracts (at 7th wk of birds' life)

Traits	Groups					SEM
	NC	GR	GC	O	C	
Total weight (g)	9.79	12.10	11.51	11.59	11.42	*0.309*
Total length (cm)	79.95^{c}	85.96^{bc}	91.53^{ab}	93.22^{a}	88.26^{ab}	*0.934*
Pancreas weight (g)	0.387^{b}	0.540^{a}	0.452^{ab}	0.483^{ab}	0.533^{a}	*0.015*
pH gizzard	5.53^{a}	5.11^{ab}	4.84^{b}	4.77^{b}	4.63^{b}	*0.079*
pH small intestine	6.24	6.22	6.19	6.21	6.07	*0.038*
pH large intestine	6.51^{a}	6.49^{ab}	6.54^{a}	6.55^{a}	6.29^{b}	*0.024*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

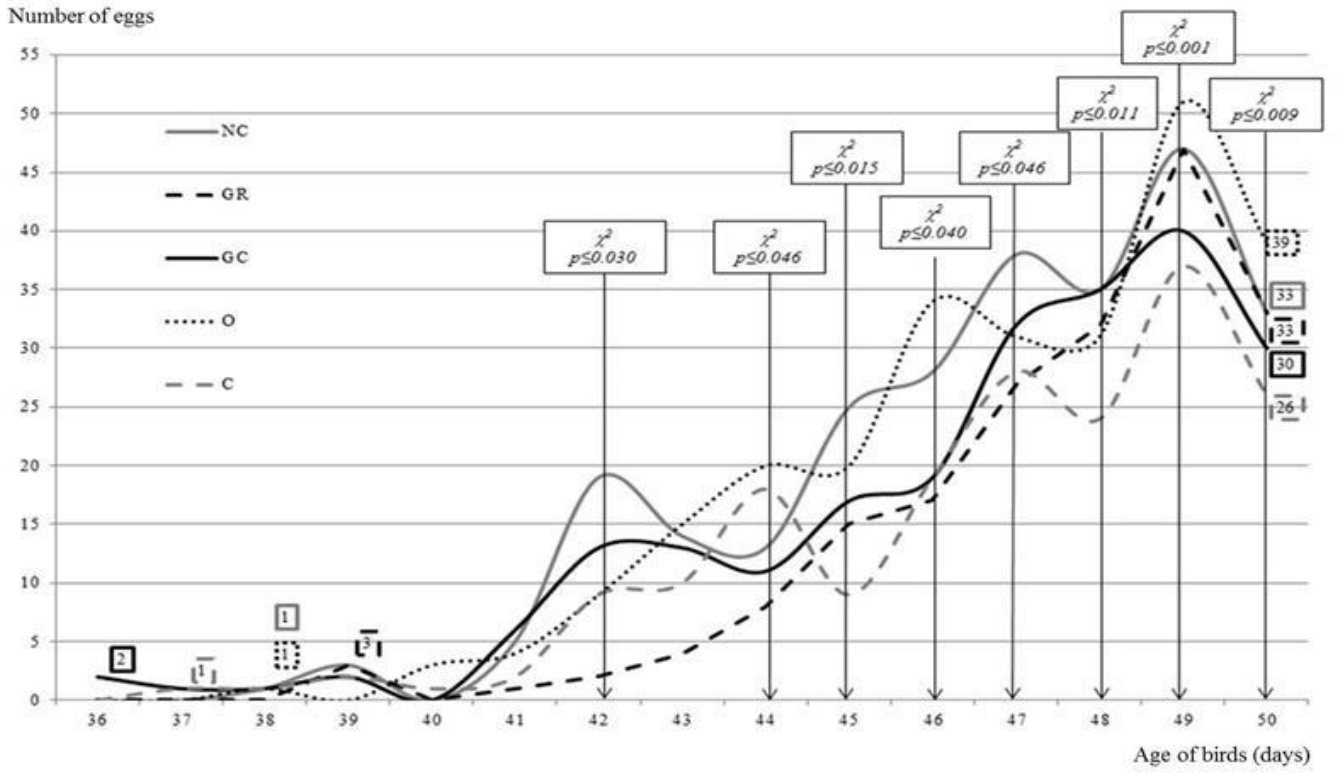
Table 9. Duodenal histomorphology of Japanese quails influenced by influenced by *in ovo* injection with aqueous solutions of plant extracts (at 7th wk of birds' life)

Traits	Groups					SEM
	NC	GR	GC	O	C	
Villus height (μm)	407.1^{b}	405.9^{b}	553.3^{a}	626.9^{a}	617.4^{a}	*15.05*
Villus width (μm)	85.7	85.3	92.8	87.1	97.0	*1.960*
Crypt depth (μm)	83.4	82.1	86.4	84.3	90.3	*2.190*
Crypt width (μm)	46.9^{ab}	42.0^{b}	52.6^{a}	50.9^{ab}	51.1^{ab}	*1.192*
Villus height /crypt depth	5.36^{b}	5.26^{b}	6.62^{ab}	7.79^{a}	7.09^{ab}	*0.260*
Villus surface area ($\times 10^3 \mu m^2$)	114.6^{b}	108.7^{b}	160.7^{a}	170.9^{a}	187.5^{a}	*6.089*
Muscular layer thickness (μm)	75.53	79.67	79.31	86.36	87.51	*1.819*
Total mucosal thickness (μm)	490.5^{b}	488.0^{b}	639.7^{a}	711.3^{a}	707.7^{a}	*15.46*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

In duodenal histomorphology, the stimulative influence of GC, O and C injected *in ovo* is visible (table 9). The biggest significant differences in villus height, villus surface area and total mucosal thickness were recorded in these groups.

It was illustrated in graph 1 that females of GC and C achieved sexual maturity earlier than other groups on 36th and 37th day of post–hatch respectively. O, NC and GR groups reached sexual maturity on 38th, 38th and 39th day respectively. The biggest number of eggs up to 50th day of birds' life was collected in O group, however, the number of eggs depended significantly on group from 42nd till 50th day of experiment.



Graph1. Sexual maturity and number of collected eggs influenced by *in ovo* injection with aqueous solutions of plant extracts (NC - negative control, GR – ginger, GC – garlic, O – oregano, C – cinnamon)

Table 10. Selected hematological traits and plasma antioxidant indices influenced by *in ovo* injection with aqueous solutions of plant extract (at 7th wk of birds' life)

Traits	Groups					SEM
	NC	GR	GC	O	C	
PCV (%)	41.50	41.19	42.19	41.56	39.50	*0.362*
Hgb (g/dl)	11.69	11.06	11.31	12.13	10.96	*0.160*
WBC ($\times 10^3/mm^3$)	14.20	14.87	12.69	17.57	14.62	*0.036*
RBC ($\times 10^6/mm^3$)	3.53	3.41	3.71	3.36	3.41	*0.059*
H/L	0.375	0.389	0.380	0.377	0.365	*0.019*
MCV (μm^3)	118.2	121.4	114.7	123.8	116.3	*2.047*
MCH (pg)	33.34^{ab}	32.54^{ab}	30.73^{b}	36.91^{a}	32.25^{ab}	*0.722*
MCHC (g/dl)	28.16^{ab}	26.85^{b}	26.83^{b}	29.17^{a}	27.68^{ab}	*0.273*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

Table 10 shows blood parameters of Japanese quails. No differences among groups in almost all investigated blood traits were stated. Blood plasma parameters are presented in Table 11. There is showed the lack of significant differences among groups with respect to CHOL, TG, GLU, TP, GGT, ALP and LDH levels. Some differences were stated in case of NEFA, AST, ALT and CREAT. It was visible that each plant extract had various effect on plasma biochemistry. For

example: low NEFA was registered in GC group; the biggest concentration of AST and ALT was stated in the groups injected with oregano, garlic and cinnamon. Also, cinnamon injection increased CREAT.

Table 11. Selected plasma biochemical profile influenced by *in ovo* injection with aqueous solutions of plant extract (at 7th wk of birds' life)

Traits	Groups					SEM
	NC	GR	GC	O	C	
CHOL (mmol/l)	2.70	2.68	2.71	2.78	2.71	*0.034*
TG (mmol/l)	0.805	0.733	0.706	0.759	0.778	*0.018*
NEFA (µmol/l)	634^{a}	637^{a}	519^{b}	596^{ab}	651^{a}	*14.55*
GLU (mmol/l)	10.09	9.55	9.36	9.92	9.90	*0.098*
TP (g/dl)	3.25	2.88	3.08	3.13	3.17	*0.052*
AST (U/l)	223^{b}	183^{b}	236^{b}	311^{a}	236^{b}	*12.70*
ALT (U/l)	3.19^{b}	3.63^{ab}	3.84^{a}	3.84^{a}	3.81^{a}	*0.086*
GGT (U/l)	1.72	1.78	1.74	1.76	1.68	*0.029*
ALP (U/l)	927	943	942	962	976	*8.45*
LDH (U/l)	725	777	813	679	669	*17.68*
CREAT (µmol/l)	24.2^{ab}	23.4^{ab}	22.7^{ab}	22.6^{b}	25.3^{a}	*0.365*
LOOH (µmol/l)	25.18	26.58	24.89	26.35	29.09	*3.575*
MDA (µmol/l)	1.195	1.279	1.255	1.475	1.278	*0.034*
SOD (U/mol)	284.6	280.0	291.7	286.3	283.3	*3.631*
CAT (U/mol)	163.6	141.7	134.4	160.7	151.4	*0.676*

NC – negative control, PC – positive control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ – means within rows (for groups) differ significantly at $p \leq 0.05$

Experiment III

The aim of the study was to investigate the influence of extracts of ginger (GR, *Zingiber officinale*), garlic (GC, *Allium sativum*), oregano (O, *Origanum vulgare*) and cinnamon (C, *Cinnamomum verum*) as natural phytogenic additives supplemented to laying Japanese quails diet and hydrogen peroxide (H_2O_2) in drinking water as oxidative stress factor during eggs production period. Some of productive performance traits, physiological aspects and antioxidant status of birds were evaluated.

Materials and methods

Experiment started in 9 wks of birds' life till 4 months . Birds were allocated into 10 dietary treatments (4 replicates per group) in sex ratio 4♂:17♀, according to schema of the experiment (table 12).

Table 12. The schema of experiment (number of birds)

Factors		Plant extracts					Total
		Con	GR	GC	O	C	
H_2O_2	(-)	84	84	84	84	84	420
	(+)	84	84	84	84	84	420
	Total	168	168	168	168	168	840

Addition of 0.25% of plant extracts to diet and 0.2% of hydrgen peroxide in drinking water of layer quails. Feed and water were provided for *ad libitum* and the diet was given in pelleted form to meet nutrient specification for the layer type-quail

Studied characteristics

Quails were weighed individually every 2 weeks. The feed intake and egg weight were weighed in each replicate daily and weekly respectively. Feed conversion ratio was estimated. Egg production was calculated on hen day egg production (HD%).

To investigate quality of eggs, the electronic set EQM (Egg Quality Measurements by TSS®) and Instron Mini 55 apparatus were used. The following egg traits were evaluated: features describing shell (weight, strength, and thickness), albumen (weight, height and Haugh unit), yolk (weight, colour and index).

The same method for anatomical and histomorphological procedures as in previous experiment was followed to determine morphometric profile of internal organs as well as for duodenum evaluation. The liver sample was fixed in 4% buffered formalin solution, dehydrated, and embedded in paraffin. The 4-μm-thick sections were stained with (H&E) for processing. Microscopic features of liver were indicated for histopathological evaluation using optical microscope equipped with video camera and computer with measurement scale and imaging software. Moreover, all alterations from the normal structure of liver were recognized using a suggested criteria.

The same method in previous experiment was followed to determine pH gastrointestinal tract. On slaughter day, intestinal contents were collected from different parts of gastrointestinal tract for enumerating the microbial population for total bacteria and fungi and coliform. Samples were placed in aseptic containers. From each sample, 20 g of material were collected and placed in sterile bottles containing 180 ml of Ringer's liquid. After incubation microbial colonies were counted according to norms. The results were presented as colony forming units per 1 g of the analysed material (CFU/g). To identify bacteria, fungi and molds colonies were evaluated by

macroscopic way, colorated with Gram's staining and then sieved by reduction. The final identification was done on the basis of biochemical API tests.

The blood and serum were evaluated by methods used in previous experiment. The same parameters of hatchability were also estimated.

The data were analysed with the use of the statistical package SPSS 20.0PL. The Kolmogorov–Smirnov test was carried out in case of normality of data. Obtained numerical data were verified by t-test and one- or two-factorial analysis of variance and Tukey's test. The statistical model incorporated both analysed factors, such as type of plant and the use of hydrogen peroxide (H_2O_2), interaction between these factors were compiled as well. The significance level was defined as 5% ($p \leq 0.05$). The χ^2 test was used to analyse non-parametric data of experiment (i.e. mortality and hatchability).

Results

In table 13, the body weight of birds is presented. Plant extracts supplementation contributed to obtain similar body weight in total mean. A decrease of body weight caused by hydrogen peroxide additive was visible. The value of daily feed intake was equal under effect of plant extract. Considerably, the addition of H_2O_2 caused significant lowering in feed intake in total mean. Feed conversion ratio was reduced significantly in all dietary plant extracts in total mean. No differences were also observed in interactions between each group of plant extract with or without H_2O_2. The total effect of H_2O_2 reduced egg production in total mean. The positive influence of plant extracts supplementation was demonstrated by all groups in total mean of egg weight. However, oxidative stress caused by H_2O_2 decreased significantly egg weight. The best survivability was registered in GR group (3rd), it amounted to 100%. Strong antioxidative properties of oregano and cinnamon extracts are also visible. Plant extracts, except garlic, regardless of hydrogen peroxide additive contributed to an increase in birds' survivability.

Table 13. Total mean of productive performance of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
Body weight (g)	(-)	187.4^{a}	182.5^{ab}	180.0^{abcd}	185.9^{b}	181.2^{abc}	183.4*	*0.613*
	(+)	174.8^{bcd}	173.7^{cd}	173.7^{cd}	172.0^{d}	175.2^{bcd}	173.9	
	Total	181.1	178.1	176.9	178.9	178.2		
Daily feed intake (g)	(-)	26.18^{abcd}	27.04^{ab}	27.12^{a}	26.78^{abc}	27.06^{a}	26.84*	*0.179*
	(+)	24.79^{bcde}	24.75^{cde}	24.12^{de}	23.35^{e}	24.06^{de}	24.21	

	Total	25.48	25.89	25.62	25.07	25.56		
Feed conversion ratio (g feed/g egg)	(-)	3.06^{b}	2.72^{cd}	2.68^{cd}	2.98^{bc}	2.65^{c}	2.82	*0.028*
	(+)	3.56^{a}	2.67^{cd}	2.78^{bcd}	2.83^{bcd}	2.61^{c}	2.89	
	Total	3.31^{a}	2.69^{c}	2.73^{c}	2.90^{b}	2.63^{c}		
Egg production (HD) (%)	(-)	80.39^{de}	88.08^{ab}	90.36^{a}	81.13^{cde}	90.32^{a}	86.05*	*0.599*
	(+)	68.73^{f}	86.91^{abc}	$82.38b^{cde}$	76.62^{e}	85.91^{abcd}	80.11	
	Total	74.56	87.49	86.37	78.87	88.11		
Egg weight (g)	(-)	10.68^{b}	11.52^{a}	11.41^{a}	11.30^{a}	11.48^{a}	11.10*	*0.019*
	(+)	10.22^{c}	10.83^{b}	10.77^{b}	10.92^{b}	10.89^{b}	10.83	
	Total	10.45^{b}	11.18^{a}	11.09^{a}	11.11^{a}	11.19^{a}		
Total mortality (%)	(-)	4.76^{ab}	0.00^{b}	3.17^{ab}	3.17^{ab}	1.59^{b}	2.54	*0.625*
	(+)	7.94^{a}	3.17^{ab}	3.17^{ab}	1.59^{b}	1.59^{b}	3.49	
	Total	6.35^{a}	1.59^{b}	3.17^{ab}	2.38^{b}	1.59^{b}		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

In table 14, traits of egg yolk are presented. In total, all plant extracts increased yolk colour in total mean. Also, total mean of yolk index was increased by GR and C groups. However, it is clearly visible that H_2O_2 considerably decreased the values of yolk index, colour and weight in total mean.

Table 14. Total mean of yolk egg quality of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
Yolk index	**(-)**	45.25^{bc}	47.64^{a}	46.82^{abc}	46.37^{abc}	47.72^{a}	46.77*	*0.143*
	(+)	46.15^{abc}	46.81^{abc}	46.12^{abc}	44.82^{c}	47.19^{ab}	46.18	
	Total	45.69^{b}	47.22^{a}	46.46^{b}	45.55^{ab}	47.46^{a}		
Yolk colour (pts)	**(-)**	10.26^{ef}	11.15^{bc}	10.50^{de}	11.77^{a}	11.57^{ab}	11.05*	*0.046*
	(+)	9.72^{f}	10.68^{cde}	10.31^{ef}	11.47^{ab}	11.04^{bcd}	10.65	
	Total	9.99^{d}	10.92^{b}	10.40^{c}	11.62^{a}	11.31^{a}		
Yolk weight (g)	**(-)**	3.39	3.56	3.35	3.21	3.64	3.43*	*0.040*
	(+)	3.08	3.11	3.20	3.09	3.25	3.14	
	Total	3.24	3.34	3.28	3.15	3.44		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

In table 15, the egg albumen quality traits are shown. In general the effect of plant extracts, the O group had the highest values in Haugh unit. General effect of H_2O_2 increased Haugh unit in total mean but reduced the albumen weight in total mean. In the interaction among groups, no differences were observed between each dietary group with or without H_2O_2.

Table 15. Total mean of albumen egg quality of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
Albumen height (mm)	(-)	3.86	3.86	4.19	3.97	4.00	3.97	*0.075*
	(+)	4.04	4.06	4.33	4.28	4.03	4.15	
	Total	3.95	3.96	4.26	4.13	4.01		
Haugh's unit	(-)	84.6	85.6	86.1	86.6	86.7	85.9	*0.234*
	(+)	87.0	87.5	87.4	87.7	87.3	87.4*	
	Total	85.8	86.5	86.8	87.2	87.0		
Albumen weight (g)	(-)	6.04	6.12	6.26	6.36	6.01	6.16*	*0.044*
	(+)	5.78	5.88	5.75	6.20	5.87	5.90	
	Total	5.91	6.00	6.01	6.28	5.94		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

Table 16. Total mean of egg shell quality of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
Shell strength (N)	(-)	15.60^{c}	17.51^{a}	16.71^{abc}	16.91^{abc}	17.26^{ab}	16.80*	*0.055*
	(+)	15.87^{bc}	15.69^{bc}	15.90^{abc}	16.12^{abc}	16.05^{abc}	15.93	
	Total	15.73	16.60	16.30	16.52	16.65		
Shell thickness (mm)	(-)	0.181^{abc}	0.188^{a}	0.182^{ab}	0.180^{abc}	0.173^{bc}	0.181*	*0.001*
	(+)	0.182^{abc}	0.178^{abc}	0.178^{abc}	0.176^{bc}	0.172^{c}	0.177	
	Total	0.181^{a}	0.183^{a}	0.180^{a}	0.178^{b}	0.173^{b}		
Shell weight (g)	(-)	1.53^{c}	1.65^{a}	1.62^{ab}	1.59^{abc}	1.61^{ab}	1.60*	*0.005*
	(+)	1.53^{c}	1.59^{abc}	1.55^{bc}	1.58^{abc}	1.57^{bc}	1.56	
	Total	1.53^{b}	1.62^{a}	1.58^{a}	1.58^{a}	1.59^{a}		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

Egg shell quality traits are summarized in table 16. All plant extracts contributed to increase shell weight. H_2O_2 additive contributed to a reduction of shell strength as well as caused a decrease

of shell thickness and shell weight. Experimental factors interaction was significant in all traits of egg shell.

The stimulative effect of all plant extracts was visible in case of all the evaluated measurements of total weight and length of gastrointestinal tract (Table 17). The biggest ovary weight was stated in GR, GC and O groups. Also, the biggest weight of this organ was noticed in GC group (without H_2O_2) and the smallest in 2nd group supplemented just with oxidative stress factor (interaction). GR, O and C groups increase oviduct length. Hydrogen peroxide had an opposite effect on these organs.

Table 17. Visceral organs profile at 16th wk of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
Total weight of gastrointestinal tract (g)	(-)	12.76^{ab}	14.32^{a}	14.00^{a}	14.13^{a}	13.87^{a}	13.81*	*0.168*
	(+)	10.91^{b}	12.28^{ab}	12.90^{ab}	12.92^{ab}	12.38^{ab}	12.28	
	Total	11.84^{b}	13.26^{a}	13.45^{a}	13.53^{a}	13.13^{ab}		
Total length of gastrointestinal tract (cm)	(-)	92.2^{bcd}	99.8^{ab}	99.4^{ab}	99.9^{a}	98.7^{ab}	98.0*	*0.623*
	(+)	86.3^{d}	93.6^{abcd}	94.8^{abc}	91.3^{cd}	96.6^{abc}	92.5	
	Total	89.3^{b}	96.6^{a}	97.1^{a}	95.6^{a}	97.7^{a}		
Ovary weight (g)	(-)	4.10^{bc}	6.04^{ab}	6.30^{a}	5.97^{ab}	5.01^{abc}	5.50*	*0.181*
	(+)	3.18^{c}	5.16^{abc}	4.12^{bc}	4.90^{abc}	4.24^{abc}	4.32	
	Total	3.64^{b}	5.60^{a}	5.21^{a}	5.43^{a}	4.56^{ab}		
Oviduct length(cm)	(-)	29.00^{ab}	32.22^{a}	30.62^{ab}	31.02^{ab}	32.70^{a}	31.06	*0.338*
	(+)	27.02^{b}	31.05^{ab}	29.18^{ab}	31.85^{a}	30.48^{ab}	29.93	
	Total	28.01^{b}	31.63^{a}	29.90^{ab}	31.43^{a}	31.40^{a}		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

Duodenal histomorphology is presented in (table 18). In comparison to the total effect of control groups, all dietary plant extracts groups registered the highest values in villus height, villus height/crypt depth, muscular layer thickness. The villus surface area was the highest in birds from groups supplemented with GC, O and C. Oxidative stress resulted from use of H_2O_2 reduced considerably the villus height, villus height/crypt depth and villus surface area. Interaction of experimental factors was significant in case of villus height, villus height/crypt depth, villus surface area and muscular layer thickness.

Table 18. Duodenal histomorphology at 16th wk of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
Villus height (μm)	(-)	610.9^{c}	646.6^{c}	789.4^{a}	720.9^{ab}	729.8^{ab}	700.0*	*8.837*
	(+)	441.4^{d}	641.9^{c}	610.9^{c}	656.2^{bc}	645.4^{c}	598.4	
	Total	523.2^{b}	644.4^{a}	703.2^{a}	688.6^{a}	687.6^{a}		
Villus height/crypt depth	(-)	6.52^{a}	7.42^{a}	8.11^{a}	7.67^{a}	8.07^{a}	7.57*	*0.141*
	(+)	4.62^{b}	7.82^{a}	7.24^{a}	7.13^{a}	7.60^{a}	6.87	
	Total	5.54^{b}	7.61^{a}	7.69^{a}	7.40^{a}	7.84^{a}		
Villus surface area ($\times 10^3 \mu m^2$)	(-)	182.6^{c}	185.5^{bc}	265.7^{a}	218.4^{abc}	234.6^{ab}	217.4*	*8.088*
	(+)	123.2^{d}	173.3^{cd}	178.1^{c}	190.0^{bc}	187.4^{bc}	170.2	
	Total	151.8^{c}	179.8^{bc}	223.4^{a}	204.2^{ab}	211.0^{ab}		
Muscular layer thickness (μm)	(-)	86.1^{a}	97.3^{a}	87.6^{a}	91.3^{a}	88.2^{a}	90.2	*1.265*
	(+)	66.8^{b}	90.8^{a}	92.4^{a}	86.1^{a}	95.5^{a}	86.2	
	Total	76.2^{b}	94.2^{a}	89.9^{a}	88.7^{a}	91.9^{a}		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

Table 19. Histopathological changes in liver at 16th wk of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Findings	H_2O_2	Groups				
		Con	GR	GC	O	C
Fat droplets	(-)	+++	–	–	–	–
	(+)	+++	–	–	++	+
Congestive hemorrhages	(-)	–	–	–	–	–
	(+)	+++	+	–	–	–
Kupffer cells	(-)	++	–	+	++	+
	(+)	+++	+	++	+	+
Cells necrosis	(-)	+	–	–	–	–
	(+)	+++	–	–	–	++

Con – control, GR– ginger, GC – garlic, O – oregano, C – cinnamon
– no change was found in all birds in group
+ a change was rare in all birds in group
++ a change was relatively common in all birds in group
+++ a change was very often found in all birds in group

Table 19 illustrates histopathological changes in liver of Japanese quail females. These examinations on the treated groups exhibited remarkable pathological changes in liver tissue in response to oxidative stress caused by H_2O_2 in drinking water which were represented by fat droplets, congestive hemorrhages, veins dilation, Kupffer cells, leukocytes infiltration and cells

necrosis. Inclusion of particular plant extracts in diet reduced these abnormal incidents. In case of positive mode of action, the most effective was GC and GR extracts followed by O and C extracts.

Table 20. Acidity and microbial count (Log10 CFU/g) of gastrointestinal tract at 16 th wk of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
pH gizzard	(-)	5.87^{a}	4.65^{c}	5.37^{abc}	5.84^{a}	4.85^{bc}	5.32	*0.063*
	(+)	5.91^{a}	5.22^{abc}	5.49^{ab}	5.78^{a}	5.46^{ab}	5.57*	
	Total	5.89^{a}	4.94^{c}	5.43^{ab}	5.81^{a}	5.16^{bc}		
Total number of bacteria	(-)	6.44^{bc}	4.44^{d}	9.44^{a}	7.95^{ab}	5.38^{cd}	6.73*	*0.413*
	(+)	2.81^{e}	1.98^{e}	8.88^{a}	5.66^{cd}	2.52^{e}	4.37	
	Total	4.62^{c}	3.21^{c}	9.16^{a}	6.80^{b}	3.95^{c}		
Coliforms	(-)	3.58^{c}	3.21^{cd}	4.75^{a}	3.10^{de}	3.60^{c}	3.65*	*0.121*
	(+)	2.80^{e}	2.18^{g}	4.08^{b}	2.76^{ef}	2.39^{fg}	2.84	
	Total	3.19^{b}	2.70^{b}	4.41^{a}	2.93^{b}	2.99^{b}		
Total number of fungi	(-)	1.26^{ab}	0.00	0.66^{c}	1.10^{ab}	1.30^{a}	1.08	*0.487*
	(+)	0.00	0.00	0.83^{bc}	0.98^{abc}	1.23^{ab}	1.01	
	Total	0.63^{a}	0.00	0.74^{b}	1.04^{a}	1.26^{a}		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

In table 20 shows a reduction in pH level of gizzard was noticed in groups without H_2O_2 and, at the same time, in GR and C groups it was significantly lower value compared to Con. In the total number of bacteria evaluated in gastrointestinal tract content, the GR and C groups did not differ from Con. A high total number of bacteria was stated in GC and O extracts. In groups of GR, O and C did not differ from Con in the number of coliforms. A total reduction of fungi was stated in GR group. H_2O_2 caused a significant decrease of total number of bacteria and coliforms. All microbial parameters stayed under the influence of a two-factorial interaction.

In table 21 hatching results are presented. The main effect of H_2O_2 was significant ($p \leq 0.000$) to increase crippled chicks and this ratio was reduced ($p \leq 0.052$) by GC, O and C. H_2O_2 reduced significantly body weight of hatchlings, at the same time, the biggest hatched chicks derived from eggs of O group.

Table 21. Chosen hatching results at 8 wk of Japanese quail influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Trait	H_2O_2	Groups						χ^2 (*p-value*)		
		Con	GR	GC	O	C	Total	Plant extracts	H_2O_2	Total
Crippled chicks	(-)	0.93	2.31	0.79	0.78	0.00	0.95	*0.052*	*0.000*	*0.273*
	(+)	2.26	3.20	0.79	0.74	0.00	1.39			
	Total	1.43	2.75	0.79	0.76	0.00				
Trait	H_2O_2	Groups						SEM		
		Con	GR	GC	O	C	Total			
Body weight of hatched chicks (g)	(-)	7.34^{abc}	7.47^{abc}	7.36^{abc}	7.63^{a}	7.58^{ab}	7.46*	*0.028*		
	(+)	6.85^{d}	7.28^{bc}	7.40^{abc}	7.50^{abc}	7.18^{cd}	7.24			
	Total	7.15^{b}	7.38^{ab}	7.38^{ab}	7.57^{a}	7.38^{ab}				

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

Selected traits plasma biochemical profile are presented in table 22. GLU was considerably decreased by cinnamon extract. A positive reduction was obtained in ALT and GGT activity by 12.27 and 9.65% respectively in group supplemented with GC. Ca level was reduced as well in this group. In general effect, H_2O_2 had no influence in almost all parameters. However, H_2O_2 reduced Ca level. In plasma a significant interaction of experimental factors was observed in GLU, ALT, GGT and Ca levels. The GC, O and C groups increased SOD but the same groups did not differ statistically from Con in CAT. The MDA was not changed, neither by plant extracts nor H_2O_2. However, interaction between both experimental factors was significant in case of SOD and CAT.

Table 22. Selected traits of plasma biochemical profile of Japanese quail (16^{th} wks. of age) influenced by dietary inclusion of plant extracts and oxidative stress factor (hydrogen peroxide) in drinking water

Traits	H_2O_2	Groups						SEM
		Con	GR	GC	O	C	Total	
HDL (mmol/l)	**(-)**	0.92	0.72	0.81	0.97	0.97	0.87	*0.020*
	(+)	0.72	0.98	0.87	0.74	0.94	0.85	
	Total	0.82	0.85	0.84	0.86	0.95		
LDL (mmol/l)	**(-)**	1.73^{ab}	1.62^{ab}	1.75^{ab}	1.68^{ab}	1.62^{ab}	1.68	*0.012*
	(+)	1.62^{ab}	1.73^{ab}	1.77^{a}	1.60^{b}	1.65^{ab}	1.67	
	Total	1.67^{ab}	1.67^{ab}	1.76^{a}	1.64^{b}	1.63^{b}		
NEFA	**(-)**	595.6^{ab}	640.2^{ab}	566.9^{ab}	607.2^{ab}	565.5^{ab}	594.9	*9.450*

(µmol/l)	(+)	579.6^{ab}	688.3^{a}	606.6^{ab}	586.7^{ab}	528.4^{b}	599.8	
	Total	587.6^{ab}	664.3^{a}	586.8^{ab}	597.3^{ab}	548.0^{b}		
GLU (mmol/l)	(-)	9.53^{abc}	9.17^{bc}	9.96^{ab}	9.52^{abc}	9.38^{abc}	9.51	*0.064*
	(+)	9.90^{ab}	9.61^{abc}	10.13^{a}	9.24^{bc}	8.91^{c}	9.58	
	Total	9.71^{ab}	9.39^{bc}	10.05^{a}	9.38^{bc}	9.16^{c}		
ALT (U/l)	(-)	3.75	3.91	3.36	3.78	3.56	3.67	*0.041*
	(+)	3.83	3.79	3.36	3.68	3.55	3.62	
	Total	3.83^{a}	3.79^{a}	3.36^{b}	3.68^{ab}	3.55^{ab}		
GGT (U/l)	(-)	1.79^{ab}	1.83^{a}	1.56^{c}	1.60^{bc}	1.56^{c}	1.67	*0.016*
	(+)	1.73^{abc}	1.60^{bc}	1.63^{abc}	1.67^{abc}	1.79^{ab}	1.68	
	Total	1.76^{a}	1.71^{ab}	1.59^{b}	1.63^{ab}	1.67^{ab}		
ALP (U/l)	(-)	979.2	1025.1	979.3	933.9	965.6	977.1	*7.270*
	(+)	958.8	1027.4	997.1	986.3	945.1	983.8	
	Total	969.0^{ab}	1026.3^{a}	988.2^{ab}	959.3^{b}	955.9^{b}		
Ca (mmol/l)	(-)	9.14^{ab}	8.68^{ab}	8.12^{ab}	8.53^{ab}	9.35^{a}	8.77*	*0.117*
	(+)	9.11^{ab}	8.42^{ab}	7.54^{b}	8.75^{ab}	8.49^{ab}	8.46	
	Total	9.12^{a}	8.55^{ab}	7.83^{b}	8.64^{ab}	8.95^{a}		
MDA (µmol/l)	(-)	0.350	0.465	0.405	0.590	0.485	0.459	*0.129*
	(+)	1.545	0.750	0.410	0.745	0.635	0.817	
	Total	0.948	0.608	0.408	0.668	0.560		
SOD (U/mol)	(-)	194.3^{de}	190.7^{e}	282.5^{abcd}	265.2^{abcde}	313.6^{ab}	249.2	*8.346*
	(+)	218.2^{cde}	236.2^{bcde}	338.2^{a}	286.8^{abc}	287.1^{abc}	273.3	
	Total	206.2^{b}	213.5^{b}	310.3^{a}	276.0^{a}	300.3^{a}		
CAT (U/mol)	(-)	837.6^{a}	420.9^{bc}	479.6^{bc}	699.0^{ab}	407.9^{c}	569.0	*24.116*
	(+)	514.9^{bc}	531.6^{bc}	523.5^{bc}	641.1^{abc}	565.4^{abc}	555.3	
	Total	676.2^{a}	476.3^{b}	501.5^{ab}	670.1^{ab}	486.6^{ab}		

Con – control, GR – ginger, GC – garlic, O – oregano, C – cinnamon; $^{a, b}$ - means within rows (for plant extracts) or in frame (for interactions) differ significantly at $p \leq 0.05$, * - means within columns (for H_2O_2) differ significantly at $p \leq 0.05$

Conclusions

The obtained results based on suggested hypotheses with respect to using selected plant extracts (ginger, garlic, oregano and cinnamon) as biological and natural materials in quail productivity, it could be summarized as follows:

1. All disinfectants prepared from aqueous extracts of plants maintained the hatchability and in post hatch productivity of chicks. Antimicrobial activity on eggshell was positively influenced by ginger and garlic extracts. These safe disinfectant solutions may be recommended to be used instead of harmful formaldehyde gas for hatching eggs disinfection.
2. Birds from all groups injected *in ovo* with plant extracts solutions had better or stable productive and physiological status in relation to non-injected birds, depending on determined extract.
3. All plant extracts supplemented in diet of layer quail alleviated oxidative stress status induced artificially by hydrogen peroxide in drinking water. It was reflected in improved body weight, feed efficiency ratio and eggs production as well as egg quality traits. The stability of most haematological and biochemical parameters as well as oxidative biomarkers, despite of applicated oxidative factor, may point at the ability of plant extracts to neutralize oxidative stress effects, however their efficacy depends on plant species.

The obtained results indicated that plant extracts may be useful in chosen aspects of poultry production, however, their efficacy in all experiments was in a species-dependent manner. These facts justified the continuation of further investigations, especially, to determine the exact effective dose also with regard to application on other birds species.