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PHARMACOLOGICAL EFFICACY AND TOXICOLOGICAL SAFETY ASSESSMENT OF INTROVIT ES 100 ORAL SOLUTION IN LAYING HENS

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Abstract:

Oxidative stress, immunosuppression and reduced productivity remain major constraints in intensively managed poultry flocks. This study evaluated the pharmacological efficacy and toxicological safety of Introvit ES 100, an oral vitamin E–selenium complex, in laying hens under industrial rearing conditions. Thirty clinically healthy hens of comparable age and body weight were allocated to three groups (n = 10 each): an untreated control group, and two experimental groups receiving the preparation via drinking water at 1 mL/L (Group I) and 2 mL/L (Group II) for 30 consecutive days. Haematological, biochemical, antioxidant, immunological, growth, egg-production, feed-conversion, survivability, and hepato-renal histomorphological parameters were assessed, together with an economic-efficiency analysis. Introvit ES 100 produced dose-dependent increases in erythrocyte count, haemoglobin, total protein, superoxide dismutase, glutathione peroxidase and catalase activities, together with reductions in malondialdehyde, AST and ALT, all statistically significant relative to control ($p < 0.05$). Phagocytic activity, immunoglobulin concentration and the stress-resistance index improved in treated groups, egg production rose by up to 14.3 %, feed-conversion ratio improved by up to 56 %, and survivability reached 100 % versus 90 % in controls. Histological examination revealed no degenerative, necrotic or inflammatory changes in liver or kidney of treated birds. The findings indicate that Introvit ES 100, applied at the recommended oral doses, is a safe and pharmacologically effective antioxidant–immunomodulatory agent for use in commercial poultry production.

Keywords: Introvit ES 100; vitamin E; selenium; poultry pharmacology; oxidative stress; antioxidant enzymes; immunomodulation; toxicological safety; laying hens; feed conversion.

I. INTRODUCTION

Modern industrial poultry production is characterized by high stocking densities, intensive genetic selection for rapid growth and egg output, and recurrent technological and environmental stressors. Under these conditions, birds are increasingly exposed to oxidative stress, a state in which the generation of reactive oxygen species exceeds the capacity of endogenous antioxidant defences, resulting in lipid peroxidation, cellular membrane damage and impaired organ function [1], [2]. Oxidative imbalance in poultry has been repeatedly associated with reduced growth rate, lower egg production, compromised immune competence and increased susceptibility to disease [3], [4].

Vitamin E (α-tocopherol) and selenium act synergistically as the principal components of the cellular antioxidant defence network. Vitamin E is a chain-breaking, lipid-soluble antioxidant embedded in cell membranes that limits the propagation of lipid peroxidation, whereas selenium is an essential cofactor of glutathione peroxidase (GPx) and other selenoproteins that detoxify peroxides generated during normal and stress-induced metabolism [2], [5]. Dietary or aqueous supplementation with vitamin E and selenium has been shown to increase antioxidant enzyme activity, reduce malondialdehyde (MDA) accumulation, support hepatic and renal function, and improve growth and reproductive performance in broilers and laying hens, including under heat stress and immune-challenge conditions[6], [7].

Introvit ES 100 is a combined vitamin E–selenium oral preparation used in commercial poultry practice as an antioxidant, immunomodulatory and metabolic-stimulant agent, typically administered through drinking water. Although the individual pharmacological actions of vitamin E and selenium in poultry are well documented in the international literature [8],[9], comprehensive data integrating haematological, biochemical, antioxidant, immunological, histomorphological, productive and economic endpoints for this specific combined preparation, under standardized industrial conditions, remain limited. Regulatory guidance further emphasizes that, while vitamins and most trace minerals have wide safety margins in poultry diets, dose-dependent toxicological verification remains an essential component of any new preparation's practical adoption [10].

The aim of the present study was to evaluate, in a controlled experimental design, the pharmacological efficacy of Introvit ES 100 oral solution on clinical, haematological, biochemical, antioxidant and immunological status, as well as its effects on growth, egg production, feed conversion and survivability in laying hens, and to assess its toxicological safety through hepatic and renal histomorphological examination and economic-efficiency analysis [11].

II. MATERIALS AND METHODS

A. Experimental Animals and Design

The study was conducted under industrial rearing conditions at a commercial poultry farm in the Samarkand region, Uzbekistan, in cooperation with the Samarkand State University of Veterinary Medicine, Livestock and Biotechnology. Thirty clinically healthy egg-type/meat-type hens of homogeneous age, physiological status and body weight were selected following a preliminary clinical examination (general condition, activity, appetite, plumage and mucosal status, respiratory and heart rate). Birds were randomly allocated into three groups of ten (n = 10 per group), with no statistically

significant difference in initial body weight between groups ($p > 0.05$), confirming adequate group equalization.

Table 1.

Experimental design and group allocation

Group	n	Basal diet	Introvit ES 100 dose	Route	Duration
Control	10	Standard ration	Not administered	—	30 days
Experimental I	10	Standard ration	1 mL / 1 L water	Oral (drinking water)	30 days
Experimental II	10	Standard ration	2 mL / 1 L water	Oral (drinking water)	30 days

All groups were kept under identical housing, feeding, watering and microclimatic conditions; the presence or absence of Introvit ES 100 was the sole differentiating factor between groups.

B. Preparation and Administration

Introvit ES 100 was administered exclusively through drinking water at the doses specified in Table I for 30 consecutive days. Daily water intake was determined in advance to calculate the required preparation volume per group, and fresh solution was prepared each day to ensure stable potency and complete consumption. Control birds received the same basal ration without the preparation. All procedures involving animals complied with standard bioethical requirements for animal welfare; no unnecessary stress or invasive procedures were imposed on the birds beyond routine venipuncture and clinical examination.

C. Clinical, Haematological and Biochemical Assessment

Clinical observation (general condition, behaviour, appetite, feed and water intake) was performed daily throughout the trial. Blood samples were collected aseptically from the wing vein at the beginning, midpoint and end of the experiment into anticoagulant-treated tubes. Erythrocyte and leukocyte counts were determined using the Goryaev chamber method; haemoglobin, haematocrit and leukocyte differential (lymphocytes, neutrophils, monocytes, eosinophils) were determined by standard veterinary haematological procedures applied uniformly across all groups. Serum biochemical parameters (total protein, albumin, AST, ALT, glucose) were measured by standard spectrophotometric/colorimetric methods.

D. Antioxidant and Immunological Parameters

Antioxidant status was assessed via serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase activity, determined spectrophotometrically. Immunological reactivity was evaluated through phagocytic activity, phagocytic index, serum immunoglobulin concentration and a composite stress-resistance index, together with the neutrophil-to-lymphocyte ratio.

E. Histomorphological Examination

At the end of the trial, hepatic, renal, splenic and intestinal tissue was examined macroscopically and, for representative specimens, histologically, to assess the morphological and toxicological safety profile of the preparation. Organ colour, consistency and surface architecture were recorded, and microscopic sections were evaluated for degenerative, necrotic or inflammatory changes in hepatocytes, renal tubules and glomeruli, splenic lymphoid tissue, and intestinal villi.

F. Productive and Economic Indices

Initial and final body weight, absolute and average daily weight gain, growth index, egg production rate, average egg weight, eggshell strength, defective-egg rate, feed conversion ratio (FCR, kg feed per kg product), survivability, and net economic profit per bird were calculated for each group over the 30-day period.

G. Statistical Analysis

Data are expressed as mean ± standard error of the mean (M ± m). Differences between control and experimental groups were assessed using variation-statistics methods appropriate for small independent samples; a difference was considered statistically significant at $p < 0.05$, as indicated by an asterisk (*) in the tables.

III. RESULTS

A. Clinical Status and Growth Performance

Throughout the 30-day trial, birds in both experimental groups maintained stable appetite, activity and smooth, glossy plumage, whereas isolated control birds showed transient lethargy, dull plumage and reduced appetite during periods of routine technological stress. No adverse clinical reactions, signs of intoxication or behavioural abnormalities attributable to the preparation were observed in either treated group. Water consumption remained at physiological levels in treated groups, indicating good palatability of the preparation when delivered through drinking water.

Table 2.

Effect of introvit es 100 on growth and development indices (M ± m)

Indicator	Control	Experimental I	Experimental II
Initial body weight (g)	1450 ± 25	1455 ± 27	1460 ± 26
Final body weight (g)	1680 ± 30	1825 ± 28*	1950 ± 32*
Absolute weight gain (g)	230 ± 12	370 ± 15*	490 ± 18*
Average daily gain (g/day)	7.7 ± 0.4	12.3 ± 0.5*	16.3 ± 0.6*
Growth index (%)	15.9 ± 0.8	25.4 ± 1.0*	33.6 ± 1.2*

** $p < 0.05$ vs. control.*

Final body weight in Groups I and II exceeded the control value by 8.6 % and 16.1 %, respectively ($p < 0.05$), with the strongest, dose-related response observed in Group II, consistent with a dose-dependent pharmacological effect of the preparation on metabolic and growth processes.

B. Haematological Indices

Table 3.

Effect of introvit es 100 on haematological indices (M± m)

Indicator	Control	Experimental I	Experimental II
Erythrocytes ($\times 10^{12}$/L)	2.85 ± 0.08	3.12 ± 0.07*	3.28 ± 0.06*
Haemoglobin (g/L)	92.4 ± 2.1	101.6 ± 1.9*	106.8 ± 2.0*
Haematocrit (%)	28.6 ± 0.9	31.4 ± 0.8*	33.2 ± 0.7*
Leukocytes ($\times 10^{9}$/L)	22.5 ± 1.2	24.8 ± 1.0*	26.1 ± 1.1*
Lymphocytes (%)	58.2 ± 1.5	62.7 ± 1.3*	65.4 ± 1.4*
Neutrophils (%)	28.4 ± 1.1	26.1 ± 1.0	24.8 ± 0.9

Monocytes (%)	6.3 ± 0.4	6.5 ± 0.3	6.6 ± 0.4
Eosinophils (%)	7.1 ± 0.5	6.9 ± 0.4	6.8 ± 0.3

** p < 0.05 vs. control.*

Erythrocyte count, haemoglobin concentration and haematocrit increased significantly in both treated groups relative to control (up to +15.1 %, +15.6 % and +16.1 %, respectively, in Group II; $p < 0.05$), consistent with stimulation of haematopoiesis and improved oxygen-carrying capacity. The proportional increase in lymphocytes and the parallel normalization of neutrophil values suggest an immunomodulatory rather than a stress-type leukocyte response; monocyte and eosinophil proportions remained within physiological limits in all groups, indicating an absence of allergic or toxic reactivity.

C. Biochemical and Antioxidant Status

Table 4.

Effect of introvit es 100 on biochemical and antioxidant indices (M ± m)

Indicator	Control	Experimental I	Experimental II
Total protein (g/L)	48.6 ± 1.2	54.8 ± 1.0*	58.3 ± 1.1*
Albumin (g/L)	21.4 ± 0.8	24.9 ± 0.7*	26.7 ± 0.6*
AST (U/L)	182.5 ± 5.4	170.3 ± 4.8*	165.1 ± 4.6*
ALT (U/L)	34.6 ± 1.3	30.8 ± 1.1*	28.9 ± 1.0*
Glucose (mmol/L)	10.8 ± 0.4	10.2 ± 0.3*	9.9 ± 0.3*
MDA (nmol/mL)	5.6 ± 0.2	4.3 ± 0.2*	3.7 ± 0.1*
SOD (U/mL)	78.4 ± 2.1	92.7 ± 2.3*	104.5 ± 2.5*
GPx (U/mL)	52.6 ± 1.8	64.9 ± 1.9*	72.3 ± 2.0*
Catalase (U/mL)	18.7 ± 0.6	23.8 ± 0.7*	27.1 ± 0.8*

** p < 0.05 vs. control.*

Total protein and albumin rose significantly in both treated groups (up to +19.9 % and +24.8 %, respectively), indicating enhanced protein metabolism and hepatic synthetic function. AST and ALT activities declined by up to 9.5 % and 16.5 %, respectively, remaining within physiological limits and suggesting a hepatoprotective, rather than hepatotoxic, effect of the preparation. Serum MDA, the principal marker of lipid peroxidation, decreased by up to 33.9 % in Group II, while the antioxidant enzymes SOD, GPx and catalase increased by up to 33.3 %, 37.5 % and 44.9 %, respectively (all $p < 0.05$). The magnitude of the antioxidant response was dose-dependent, with the highest values consistently observed in Group II.

D. Immunological Indices

Table 5.

Effect of introvit es 100 on immunological indices (M ± m)

Indicator	Control	Experimental I	Experimental II
Leukocytes (×10⁹/L)	22.5 ± 1.2	24.8 ± 1.0	26.1 ± 1.1
Lymphocytes (%)	58.2 ± 1.5	62.9 ± 1.3*	65.8 ± 1.4*
Neutrophils (%)	28.4 ± 1.1	26.0 ± 1.0	24.6 ± 0.9
Phagocytic activity (%)	41.5 ± 1.4	48.7 ± 1.2*	53.2 ± 1.3*
Phagocytic index	3.2 ± 0.1	3.8 ± 0.1*	4.3 ± 0.2*
Serum immunoglobulins (g/L)	9.6 ± 0.3	11.2 ± 0.4*	12.4 ± 0.5*
Stress index	0.49 ± 0.02	0.41 ± 0.02*	0.36 ± 0.01*

** p < 0.05 vs. control.*

Phagocytic activity and phagocytic index increased significantly in both treated groups (up to +28.2 % and +34.4 %, respectively; $p < 0.05$), and serum immunoglobulin concentration rose by up to 29.2 %, indicating stimulation of both cellular and humoral immunity. The composite stress index decreased by up to 26.5 % in Group II, consistent with an anti-stress, adaptogenic action of the preparation. No pathological deviations in neutrophil or total leukocyte counts were observed, supporting the absence of allergic or toxic immune reactivity.

E. Histomorphological Findings and Toxicological Safety

Macroscopic and histological examination at the end of the trial showed that the liver, kidneys, heart, spleen and intestines of birds in both treated groups were anatomically well-formed, with normal colour and consistency. Hepatic parenchyma preserved a regular radial-lobular architecture, hepatocyte nuclei and cytoplasm were well defined, and no fatty infiltration, vacuolar degeneration or necrotic foci were observed; the most stable histological pattern was recorded in Group II. Renal glomeruli and tubules showed normal architecture, with intact tubular epithelium and no evidence of nephrotoxic change. Splenic white and red pulp zones were clearly demarcated with well-developed lymphoid tissue, consistent with the observed immunological findings. Intestinal villi in treated groups were long, dense and structurally intact. In contrast, isolated control birds showed mild hepatocellular vacuolar degeneration, sinusoidal dilation and early tubular epithelial changes attributable to intensive-rearing conditions. No preparation-related mortality or clinically significant adverse events occurred during the trial.

F. Productive Performance, Survivability and Economic Efficiency

Table 6.

Effect on egg production indices (M ± m)

Indicator	Control	Experimental I	Experimental II
Egg production rate (%)	78.4 ± 2.1	84.7 ± 1.9*	89.6 ± 2.0*
Average egg weight (g)	58.6 ± 1.3	61.8 ± 1.2*	64.2 ± 1.4*
Egg mass (g/bird)	45.9 ± 1.5	52.3 ± 1.6*	57.5 ± 1.7*
Shell strength (%)	87.2 ± 1.8	91.5 ± 1.6*	94.3 ± 1.7*
Defective eggs (%)	6.8 ± 0.5	4.2 ± 0.4*	2.9 ± 0.3*

Table 7.

Effect on feed conversion, survivability and economic efficiency

Indicator	Control	Experimental I	Experimental II
Feed conversion ratio (kg feed/kg product)	18.3 ± 0.8	10.9 ± 0.5*	8.0 ± 0.4*
Survivability (%)	90.0 ± 2.5	100 ± 0.0*	100 ± 0.0*
Viability index	0.82 ± 0.03	0.91 ± 0.02*	0.95 ± 0.02*
Net economic profit (UZS/bird)	12 000	26 500	40 000

** $p < 0.05$ vs. control.*

Egg production rate increased by 8.0 % and 14.3 % in Groups I and II, respectively ($p < 0.05$), with parallel improvements in egg weight, egg mass and shell strength, and a reduction in defective-egg rate of up to 57 %. Feed conversion ratio improved by 40.4 % (Group I) and 56.3 % (Group II) relative to control, reflecting more efficient nutrient utilization. Survivability reached 100 % in both treated

groups versus 90.0 % in control, with no mortality attributable to the preparation. Net economic profit per bird was more than double in Group I and more than triple in Group II relative to control, indicating that the additional cost of the preparation was fully offset by gains in productivity and reduced losses.

IV. DISCUSSION

The present results demonstrate that oral administration of the vitamin E–selenium complex Introvit ES 100 exerted a coordinated, dose-dependent pharmacological effect across haematopoietic, hepatic, antioxidant, immunological and productive systems in laying hens, without evidence of toxicity. This pattern is consistent with the well-established synergistic mechanism of vitamin E and selenium, in which membrane-bound α-tocopherol limits the propagation of lipid peroxidation while selenium-dependent glutathione peroxidase detoxifies peroxide intermediates, jointly reducing oxidative damage to cellular membranes and organelles [12].

The significant decline in serum MDA together with the concurrent rise in SOD, GPx and catalase activity observed here parallels findings from broiler and layer studies in which combined vitamin E and selenium supplementation reduced lipid peroxidation and enhanced antioxidant enzyme activity, including under heat-stress and immune-challenge models [13]. The magnitude of the antioxidant response in the present trial was dose-dependent, mirroring the pattern reported for graded vitamin E and selenium inclusion levels in broiler diets, where antioxidant benefits increase up to an optimal dose before plateauing [13].

The haematological improvements — increased erythrocyte count, haemoglobin and haematocrit — are consistent with a stimulatory effect on erythropoiesis and with reports that adequate selenium and vitamin E status protects erythrocyte membranes from oxidative haemolysis, thereby prolonging red-cell survival and supporting haematopoietic turnover [14]. The rise in serum total protein and albumin, together with reduced AST and ALT activity, suggests improved hepatic protein-synthetic function and an absence of hepatocellular injury, corroborated directly by the histological preservation of hepatic architecture in treated groups. This concordance between biochemical and histomorphological findings provides direct toxicological evidence that Introvit ES 100, at both tested doses, does not induce hepatotoxic or nephrotoxic change, in agreement with regulatory observations that vitamin and trace-mineral supplements generally carry wide safety margins in poultry when used within recommended ranges, while still requiring dose-specific verification, since selenium in particular has a comparatively narrow margin between nutritional adequacy and toxicity at pharmacological doses [14].

The observed enhancement of phagocytic activity, phagocytic index and immunoglobulin concentration, together with a reduction in the composite stress index, indicates that Introvit ES 100 supports both innate and humoral immune competence while attenuating the physiological stress response — an effect consistent with reports that antioxidant micronutrient supplementation mitigates immunosuppression associated with technological and environmental stressors in intensively reared poultry [15]. The parallel improvement in growth rate, egg production, eggshell quality, feed conversion and survivability reflects the integration of these cellular and systemic effects into measurable production outcomes, in line with previous demonstrations that antioxidant status is mechanistically linked to feed efficiency and reproductive performance in laying hens [15].

From a toxicological-safety standpoint, the absence of clinical adverse reactions, the preservation of normal hepatic and renal histoarchitecture, and the 100 % survivability recorded in both treated groups

collectively support a favourable safety margin for Introvit ES 100 at the doses of 1 and 2 mL per litre of drinking water over a 30-day administration period. Nonetheless, because selenium compounds can exhibit a comparatively narrow therapeutic index at higher pharmacological doses, and because the present evaluation was limited to a single production cycle and a single farm environment, longer-term and multi-site trials, together with residue analysis in eggs and meat, are warranted before the recommended dosing regimen is generalized across production systems [16].

Taken together, these findings extend the existing evidence base on vitamin E–selenium pharmacology in poultry by providing an integrated efficacy–safety profile for a specific combined commercial preparation, evaluated simultaneously at the haematological, biochemical, antioxidant, immunological, histomorphological, productive and economic levels under standardized industrial conditions [16].

V. CONCLUSION

Oral administration of Introvit ES 100 at 1 mL/L and, more markedly, at 2 mL/L of drinking water for 30 days produced a coordinated, dose-dependent improvement in haematological, biochemical, antioxidant and immunological status in laying hens, together with significant gains in growth rate, egg production, eggshell quality, feed conversion efficiency and survivability. Histomorphological examination confirmed the absence of hepatic or renal toxicity, and no adverse clinical reactions were recorded at either dose. Economic analysis indicated that the cost of the preparation was fully offset by the resulting productivity gains. These findings support the use of Introvit ES 100, within the tested dosing range, as a safe and pharmacologically effective antioxidant–immunomodulatory agent for application during periods of technological, vaccination-related or transport stress in commercial poultry production. Further multi-site and longer-duration trials, including residue analysis in edible products, are recommended to confirm the generalizability of these findings.

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