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Effect of Ovirich® on Blood Chemistry in White Leghorn Layer

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ovirich® is a phytogenic molecules-yeast-mineral formulation used in poultry but still its effect on haematobiochemical parameters is unknown. Present experiment was conducted to discern the effect of Ovirich® in White Leghorn birds (90 in numbers; 22 weeks age) which were divided into three treatment groups using completely randomized design (CRD). The control group (T1) received the basal diet without any supplementation, while in treatment groups T2 and T3; the basal diet was supplemented with 0.75 kg and 1.0 kg of supplement per ton of basal feed, respectively. Blood chemistry was assessed at the end of feeding trial at 34 week age of birds. Significant differences (P≤0.05) were observed in haemoglobin, PCV, TEC, TLC, MCV, MCH, MCHC, SGPT, SGOT, total protein, albumin, globulin, glucose, total cholesterol, triglycerides, serum calcium and phosphorus in supplemented groups. It was concluded that Ovirich® in the basal diet can affect blood biochemistry in White Leghorn layers.

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Keywords: Blood chemistry; Ovirich®; whiteleghorn.

1. INTRODUCTION

Ovirich® supplement has beneficial phytogenic molecules from Capparis spinosa (Caper bush), Terminalia arjuna (Arjuna tree), Cichorium intybus (Chicory), Solanum nigrum (Black nightshade) Tamarix gallica, Achillea millefolium and Andrographis paniculata. It also contains some essential minerals like Organic Chromium, Zinc fortified with other minerals viz. (Calcium, Phosphorus, Manganese, Cobalt, Selenium) and Yeast complex. As this product effect on haematobiochemical parameters of poultry is still unknown, so this experiment was planned to assess health of bird. Earlier studies conducted on feeding of leaves of medicinal plants shows that haematological parameters are affected in poultry (Voemesse, et al, 2019, Ekezie, et al, 2023, Abdul-Moneim, et al, 2021). In this experiment effect of supplementing Ovirich® on blood parameters were studied in white leghorn.

2. MATERIALS AND METHODS

White Leghorn birds (90 in number, 22 weeks age) kept at Instructional Poultry Farm, Nagla were selected and divided into three treatment groups using completely randomized design (CRD) for feeding trial of 14 weeks. The control group (T1) received the basal diet without any supplementation, while in treatment groups T2 and T3, the basal diet was supplemented with 0.75 kg and 1.0 kg of supplement per ton of basal feed, respectively. Standard basal diets for laying birds was prepared by mixing the feed ingredients to meet the nutrient requirements of birds as per specification recommendations of BIS (2007). Proximate composition of experimental feed was analysed using AOAC (2003). Phytochemical analysis of Ovirich was conducted at the Department of Biochemistry at GBPUA&T, Pantnagar. Ovirich sample was subjected to analysis to determine their nutritional parameters like phenolic compound, amino acid content (methionine, arginine, tryptophan and lysine), total content of flavonoid, ascorbic Acid. Antinutritional factors like phytic acid and tannin were also analysed. Standard procedure was followed during biochemical analysis. Arginine was estimated by Greenstein (1961), methionine by Horn et al., (1946), Lysine by Sadasivam and Manickam (1992), tryptophan by Spies and Chambers (1949), Phenol by Bray and Thorpe (1954) Total flavonoids by Jagadish et al., *(*2009), Tannins by Kavitha and Indira (2016), Phytic acid by Haug and Lantzsch (1983) and Total anti-oxidants by Prieto et al. (1999) method.

At the end of the experiment (34 th week), around 2 ml of blood sample was collected into an EDTA-coated vial for the assessment of haematological parameters. Additionally, approximately 3 ml of blood was collected into plain vials (devoid of anticoagulant) from the wing vein. These plain vials were kept in a slanting alignment at room temperature for a period of 3 to 4 hours to facilitate the natural clotting of the blood. Once the blood had clotted, serum samples were separated by subjecting them to centrifugation at 3000 rpm for duration of 10-15 minutes. The separated serum was carefully collected into eppendorf tubes and subsequently stored at a temperature of -20°C within a deep freeze. These samples were labelled with dates and sample numbers, and were kept in this frozen state until the completion of biochemical estimations.

3. HAEMATOLOGICAL PARAMETERS

Haemoglobin: The concentration of hemoglobin (g/dl) was determined using the cyanomethemoglobin method (Drabkin and Austin, 1932).

Packed cell volume (PCV): The packed cell volume (PCV) was determined using the Microhematocrit method, following the procedure given by Martha et al. (2012).

Total erythrocytes count and total leucocyte count: The total erythrocyte count (TEC) and total leukocyte count (TLC) of the blood samples were assessed using the Hemocytometer (Neubauer's counting chamber) method, as outlined in the procedure by the author Natt and Herrick (1952).

Mean corpuscular volume: Mean corpuscular volume (MCV) was expressed in femtolitre (fl) and calculated as per following formula.

$$MCV = \frac{\text{PCV (\%)}}{\text{TEC } (106/\mu l)} x\ 10$$

Mean corpuscular haemoglobin (MCH): The mean corpuscular haemoglobin (MCH) was expressed in terms of picogram and calculated as per formula prescribed below.

$$MCH = \frac{\text{Hb (g/dl)}}{\text{TEC (106/µl)}} x\ 10$$

Mean corpuscular haemoglobin concentration (MCHC): The mean corpuscular haemoglobin concentration (g/dl) was calculated as per formula given below.

$$MCHC = \frac{\text{Hb (g/dl)}}{\text{PCV\%}}\ x\ 100$$

Serum biochemical parameters: Serum Glucose, Uric Acid, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Lactate dehydrogenase (LDH) were estimated using Erba diagnostic kit using GOD-POD Method, Uricase - PAP method, IFCC method, IFCC method and DGKC method.

Serum protein profile: The total protein and albumin concentrations in the serum were assessed through spectrophotometric analysis using the Erba Diagnostics kits. Total protein was quantified using the bromocresol green (BCG) method at a wavelength of 540 nm, and albumin was determined using the bromocresol purple (BCP) method at 630 nm.

Serum lipid profile: Cholesterol and triglycerides were measured using specific methods: CHOD-PAP for cholesterol, and GPO-PAP method for triglycerides using Diagnostic kit.

Serum calcium and serum phosphorous: The concentrations of serum calcium and phosphorus were determined using the Arsenazo III method and Ammonium Molybdate method, respectively, at 630 nm and 340 nm wavelengths, respectively, as per the manufacturer's instructions, using a standard kit supplied by ERBA Mannheim Diagnostic Centre.

Albumin Globulin ratio (A:G): Albumin globulin ratio was calculated by comparing their ratios

A:G = Concentration Of Albumin / Concentration Of Globulin

Serum LH: A CLIA (chemi luminescence immunoassay) test for Leutinising Hormone was used for estimation.

4. RESULTS AND DISCUSSION

The proximate and phytochemical composition of Ovirich® is given in Table 1. The basal diet of the experimental birds contained 18.13% crude protein and 2743.55 Kcal/Kg of Metabolizable energy.

Haematological Parameters of the white leghorn laying birds: The average values of haematological parameters presented in laying birds fed diet supplemented with Ovirich® in different treatment groups (T1, T2 and T3), at the end of supplementation period are presented in Table 2.

a) Haemoglobin (g/dl): The average blood haemoglobin(g/dl) values showed significant differences (P≤0.05) among the control(T1) and supplemented (T2 and T3) groups. Numerically, T3 had a higher value compared to T1 and T2, but no significant difference (P≥0.05) was observed betweenT2 and T3 group.

b) Packed cell volume (PCV %): The average packed cell volume values showed significant differences (P≤0.05) among the control (T1) and treatment (T2 and T3) groups. Specifically, T3 had a higher value compared to T1 and T2 group.

c) Total erythrocyte count (10^6/ mm^3): The average total erythrocyte count of T1, T2 and T3treatment groups at the end of the supplementation period showed significant differences (P≤0.05) among the control(T1) and treatment (T2 and T3) groups. Specifically, T3 had a higher value compared to T1 and T2 group.

d) Total leucocyte count (10^3/mm^3): The average total leucocyte count of T1, T2 and T3treatment groups showed significant differences (P≤0.05) among the control(T1) and treatment (T2 and T3) groups. Numerically, T3 had a lower value compared to T1 and T2 group.

e) Mean corpuscular volume (fl): The average mean corpuscular volume values showed significant differences (P≤0.05) among the control (T1) and treatment (T2 and T3) groups. T3 had a higher value compared to T1 and T2 group.

f) Mean corpuscular haemoglobin (pg) and Mean corpuscular haemoglobin concentration (g/dl): Significant differences (P≤0.05) were found in Mean corpuscular haemoglobin (pg) and Mean corpuscular haemoglobin concentration (g/dl) values among all treatment groups.

Increase in haemoglobin, Packed cell volume (PCV), Total erythrocyte count (TEC) and

Table 1. Proximate and phytochemical composition of Ovirich® supplement on % dry matter basis

S.No.	Nutrient Composition	Values
1	Dry Matter (%)	96.84
2	Crude Protein (%)	8.03
3	Ether Extract (%)	3.08
4	Crude fibre (%)	15.96
5	Total Ash (%)	37.04
6	Nitrogen free Extract (%)	35.98
7	Acid Insoluble Ash (%)	4.08
8	Total Phenol (mg GAE/g)	101.95
9	Total Flavonoid (mg QE/g)	45.80
10	Total Tannin (mg TAE/g)	80.90
11	Ascorbic Acid (mg/100gm)	115.94
12	Phytic Acid (g/100gm)	0.408
13	Methionine (g/100g)	3.624
14	Arginine (g/100g)	2.02
15	Tryptophan (g/100g)	3.22
16	Lysine (g/100g)	2.86

Table 2. Average Haematological test results of the white leghorn laying birds at the end of supplementation period of Ovirich®

Parameter	T1	T2	T3	*P*-Value
Hb (g/dl)	8.23^{b}±0.008	8.29^{a}±0.011	8.32^{a}±0.005	0.001
PCV (%)	29.38^{c}±0.04	30.24^{b}±0.04	30.96^{a}±0.008	0.00
TEC (10^{6}/ mm^{3})	2.33^{c}±0.005	2.36^{b}±0.005	2.38^{a}±0.005	0.003
TLC (10^{3}/mm^{3})	4.13^{a}±0.005	4.10^{b}±0.005	4.08^{c}±0.005	0.003
MCV (fl)	125.75^{c}±0.005	126.95^{b}±0.33	130.15^{a}±0.005	0.00
MCH (pg)	35.22^{a}±0.005	35.10^{b}±0.011	35.0^{c}±0.023	0.00
MCHC (g/dl)	28.02^{a}±0.011	27.49^{b}±0.023	26.88^{c}±0.023	0.00

Mean values bearing different superscripts (a,b,c) within a row differ significantly from each other

Mean corpuscular volume (MCV) values in Uttara chicken have been reported by Nayal et al. (2023), due to presence of different minerals like Calcium, Zinc, Aluminium, Copper, Magnesium. Haematinic property of Arjun bark and minerals have important role in the synthesis of haemoglobin. The bark contains compounds that promote the formation of red blood cells, leading to higher haemoglobin concentrations. Decrease in Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) values have also been reported by Nayal et al. (2023). Suely et al. (2016) also reported that supplementation of Arjun increases MCV, decreases MCH and MCHC.The antimicrobial property of plant used in ovirich formulation may have resisted chances of infection in experimental birds of supplemented groups.

Contrary to our results increased total leucocyte count (TLC) values have been reported by Nayal et al. (2023).

Serological parameters of laying birds: The average values of serological parameters in White Leghorn layers fed with diet supplemented with Ovirich® in different treatment groups (T1, T2 and T3), are presented in Table 3.

a) SGOT (U/L): Significant differences (P≤0.05) existed among the control (T1) and treatment (T2 and T3) groups. T3 had a lower value compared to T1 and T2 group.

Decrease in SGOT values have also been reported in Uttara chicken due to supplementation of Arjun bark (Nayal, et al., 2023).

b) SGPT (U/L): Significant differences (P≤0.05) were found among the control (T1) and treatment (T2 and T3) groups in average serum SGPT level.

Solanum nigrum extract significantly reduced the level of SGOT and SGPT, while also lowering the

generation of superoxide and hydroxyl radicals (Raju, et al., 2003**)**. Similar results have also been reported by Malisorn et al. (2020) and Nayal et al. (2023).

c) Serum Protein (g/dl): The average serum protein values differed significantly (P≤0.05) among the control(T1) and treatment (T2 and T3) groups.

Sharma et al. (2023**)** also reported that supplementation of Arjun bark powder and Sahjan leaf powder at different levels to basal diet increases total protein (g/dl) and albumin (g/dl). Inulin supplementation enhances globulin and total protein concentration (Mateova, et al., 2008).

d) Serum Albumin (g/dl): Significant differences (P≤0.05) were noted among the control (T1) and treatment (T2 and T3) groups. Numerically, T3 had a higher value compared to T1 and T2 group.

Dietary supplementation of Arjun and Sahjan increased serum albumin values in Uttara chicken (Sharma, et al., 2023).

e) Serum Globulin (g/dl): The average serum globulin values differed significantly (P≤0.05) among the control (T1) and treatment (T2 and T3) groups. Specifically, T3 had a higher value compared to T1 and T2 group.

Globulin fractions are increased due to increased protein synthesis in liver during the peak laying period (Swathi and Sudhamayee, 2005).

f) Serum Albumin Globulin Ratio (A:G): Non-significant differences (P≥0.05) were noted among the control (T1) and treatment (T2 and T3) groups.

g) Serum Triglycerides (mg/dl): Significant differences (P≤0.05) were noted among all treatment groups. Specifically, T3 had a lower value compared to T1 and T2 group.

Decrease in serum triglycerides have been reported by (Malisorn, et al., 2020**)** on feeding mixed herbal extract in hybrid ducks**.** Nayal et al. (2023) also reported decrease in serum triglycerides on feeding Arjun bark powder in Uttara layer birds.

Methanolic and phenolic extract of *Tamarix gallica* showed dose dependent decrease in the levels of cholesterol, triglyceride in hyperlipidemic rats.Phenolic extract 500 mg/kg body weight had antihyperlipidemic activity **(**Naveed, et al., 2015).

h) Serum Cholesterol (mg/dl): Significant differences (P≤0.05) were noted among the control (T1) and treatment (T2 and T3) groups. Numerically, T3 had a lower value compared to T1 and T2 groups.

Decrease in serum cholesterol level have been reported by Nayal et al. (2023). *C. spinosa* may have attributed due to its ability to reduce the activity of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase), a key enzyme in cholesterol biosynthesis (Ness, et al., 2015).

i) Serum Lactate Dehydrogenase (LDH) (U/L): No significant differences (P≥0.05) was noted among different treatment group on supplementation of supplement.

j) Uric Acid (mg/dl): The average serum uric acid level values showed significant differences (P≤0.05) among the control (T1) and treatment (T2 and T3) groups. Specifically, T3 had lower value compared to T1 and T2 group.

Sharma et al. (2023**)** reported similar results that supplementation of Arjun bark powder and Sahjan leaf powder at different levels to basal diet decreases serum uric acid levels in Uttara chicken.

k) Glucose (mg/dl): Significant differences (P≤0.05) were noted among different treatment groups in glucose values.

Nayal et al. (2023) reported similar results which indicated that supplementation of Arjun bark powder at 1g, 2g, and 4g/100gm, respectively to basal diet decreases the serum glucose in Uttara birds. Arjun bark may be responsible for stimulating B cells and elevating insulin, which increases glucose utilization in tissues and lowers glucose levels. Ragavan and Kumari, (2006) reported that oral administration of *Terminalia arjuna* in rats for 30 days significantly reduce the glucose level.

C. spinosa reduces carbohydrate absorption rates and exerts a postprandial hypoglycemic effect in the gastrointestinal tract (Lemhadri, et al., 2007).

Table 3. Average Serological test results of the laying birds at the end of supplemention period of Ovirich®

Parameters	T1	T2	T3	*P*-Value
SGOT (U/L)	182.64^{a}±0.50	175.88^{b}±1.16	171.40^{c}±0.12	0.00
SGPT (U/L)	21.6^{a}±0.19	18.67^{b}±0.13	15.77^{c}±0.04	0.00
Protein (g/dl)	4.88^{c}±0.005	4.91^{b}±0.008	4.95^{a}±0.008	0.001
Albumin (g/dl)	2.68^{c}±0.003	2.70^{b}±0.004	2.72^{a}±0.004	0.001
Globulin (g/dl)	2.196^{c}±0.002	2.21^{b}±0.003	2.23^{a}±0.003	0.001
Albumin:Globulin	1.22±0.0005	1.221±0.0004	1.219±0.0005	0.125
Triglycerides (mg/dl)	63.03^{a}±0.38	57.36^{b}±0.18	55.44^{c}±0.28	0.00
Cholesterol (mg/dl)	120.27^{a}±0.03	118.36^{b}±0.04	115.44^{c}±0.11	0.00
LDH (U/L)	220.50±2.37	218.02±2.48	217.22±3.10	0.490
Uric acid (mg/dl)	8.62^{a}±0.03	7.79^{b}±0.24	7.91^{c}±0.01	0.012
Glucose (mg/dl)	223.66^{a}±1.20	218.33^{b}±0.88	215.33^{c}±0.88	0.003
Calcium (mg/dl)	12.48^{c}±0.20	14.62^{b}±0.07	15.62^{a}±0.30	0.00
Phosphorus (mg/dl)	5.53^{c}±0.01	5.64^{b}±0.008	5.75^{a}±0.01	0.00
LH (mIU/ml)	30.28±0.57	30.33±0.60	30.35±0.54	0.996

Mean values bearing different superscripts (a,b,c) within a row differ significantly from each other

l) Calcium (mg/dl): The average serum calcium level values showed significant differences (P≤0.05) among the control(T1) and treatment (T2 and T3) groups. Specifically, T3 had a higher value compared to T1 and T2 group.

Frost and Roland (1991) reported that the level of plasma ionized Ca significantly increases in a linear manner by increasing dietary Ca levels from 2.75% to 4.25%, but not plasma total calcium.

m) Phosphorus (mg/dl): The average serum phosphorus level values showed significant differences (P≤0.05) among the control(T1) and treatment (T2 and T3) groups. Specifically, T3 had a higher value compared to T1 and T2 group.

Miles et al. (1982) reported increase in plasma phosphorus as the dietary phosphorus increased in White Leghorn Layers.

Dietary Calcium has no effect on serum phosphorus level in Hy-Line Brown layers **(**An, et al., 2016).

n) Leutinizing hormone (LH): The average LH level of the different treatment groups T1, T2 and T3 at the end of the supplementation period were non significantly different among all the treatment groups.

Due to heat stress no effect in plasma LH of White Leghoirn bird have been reported by (Rozenboim, et al., 2007). It may have happened due to fluctuations in hormone level of individual birds from which representative sample was taken.

5. CONCLUSION

It can be concluded that Ovirich can affect haemato-biochemical parameters in white leghorn layer bird. The concentration of Ovirich in poultry diet do affect blood parameters in summer month.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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