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Effect of Supplementation of HimLay® in White Leg Horn Nonlayer Diet on Egg Lay, Egg Production, Ovarian Activity, and Serum Hormone

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Abstract – *This study was designed to evaluate the effect of HimLay® on egg lay, egg production, ovarian activity, and serum LH levels in White Leghorn (WL) BV 300 nonlayer birds. A total of 20 BV 300 nonlayer birds aged 28 weeks were allocated equally into two groups viz., G1 and G2 (10 birds/group). G1 and G2 served as normal controls and the HimLay® supplemented group, respectively. Both groups were raised on normal commercial layer mash feed, and G2 was concurrently supplemented with HimLay® at a recommended mixing ratio of 500 g/ton for 8 weeks. Hen-housed egg production and pelvic bone width parameters were assessed weekly. Serum LH levels were estimated on day 0, week 4, and week 8. Ovarian activity was assessed at termination of the study. The resulting egg production data depicted that egg production was initiated from WL BV 300 nonlayer birds as early as from the first week of HimLay® treatment, and egg production was increased throughout the treatment period following supplementation of 500-g/ton HimLay®. Furthermore, almost 80% of BV 300 nonlayer birds supplemented with 500-g/ton BV 300 could produce eggs at par with BV 300 layer bird standards on week 35. Following supplementation of 500-g/ton HimLay®, serum LH levels, initiation of ovarian functions, and follicle development have improved. Supplementation of 500-g/ton HimLay® on BV 300 nonlayer birds helps in converting nonlayer birds to layer birds, and hence, usage of HimLay® could play a crucial role for the farmer of an average poultry farm to make profit.*

Keywords — *Egg production, HimLay®, Ovarian activity, Pelvic bone width, WL BV 300 nonlayer birds.*

I. INTRODUCTION

In commercial egg layer and broiler breeder operations, the birds are not usually culled after being placed in the laying house unless the birds become diseased or crippled. Profits from the average farm poultry flock could be increased if owners would study the birds and learn to tell good layers from the poor ones. First, the poultry farmer should become familiar with what to look for in the good layers, and the hens should be studied to distinguish layers from nonlayers. Farmers practice identification of poor layers and nonlayers after the laying flock has reached peak egg production or production begins to decrease, or both, since feeding and maintaining nonlayer birds become a costly affair for poultry farmers if they do not produce eggs.

The first step in selecting good layers is to determine the hens that are laying from those that are out of production. The close observer can usually make this distinction without handling the birds. The comb and wattles of the laying hen are bright red, large, full, and waxy, and the eyes are bright and of a deep color, which gives her an alert appearance. These points are easily observed in breeds, including WLs. However, the poor producer, or cull, usually has a long slender, snake-like head; dull, sunken eyes; and coarse, meaty face. The second test of a hen that is in lay is to study the condition of the pelvic bones, abdomen, and vent. In a good layer, the pelvic bones are spread wide apart and become soft, thin, and pliable. The distance between the pelvic bones and the keel bone also increases due to the increased size and length of the intestines and the enlargement of the reproductive organs. The vent is large, white, and moist. However, nonlayer hens have thick, stiff pelvic bones that are close to each other, have a heavy layer of hard fat over the abdomen, and also have a little space between the pelvic bones and the keel bone. Furthermore, the vent is wrinkled and dry [1].

The body of a hen is the factory where the eggs are made. Hence, the third test would show that an efficient producer usually has a long broad back of uniform width and a deep body. The depth of the body at the front is a very important factor in selecting a good producer. Hens with a narrow and short back and a body with a shallow depth could be culled since such hens lack the feed capacity and constitution to stand up under heavy production. Profits from the average farm poultry flock could be increased if owners would study the birds and learn to judge good layers from the poor ones [1].

Furthermore, heat stress is a major concern in modern poultry farming. Its debilitating effect on egg production is well recognized [2]. Changes in reproductive hormone secretion represent the final sequence in the neuroendocrine pathway leading to the diminished reproductive performance associated with stress. Previous studies demonstrated that stress, in a number of forms and in a number of species, increased and decreased the circulating prolactin and gonadotropins *viz* LH and FSH, respectively, in rats [3,4], cows, goats [5], turkey poults [6], laying hens [7], and turkey hens [8,9].

The use of herbs and herbal extracts as poultry feed ingredients is continuously increasing after antibiotics were banned in order to augment the production performance of layer birds. With this background, HimLay® (M/s. The Himalaya Drug Company), a polyherbal formulation developed from herbal ingredients *viz Linum usitatissimum, Asparagus racemosus, Raphanus sativus, Leptadenia reticulata, Tinospora cordifolia,* and *Lepidium sativum* claimed to possess the production performance-enhancing effects in layer birds by optimizing the functions of the female reproductive system. Hence, this study was designed and was conducted to evaluate the effect of HimLay® on egg lay, egg production, ovarian activity, and serum LH levels in WL BV 300 nonlayer birds.

II. MATERIALS AND METHODS

A. HimLay® Polyherbal Formulation

HimLay® is a proprietary polyherbal formulation developed by The Himalaya Drug Company (Bengaluru, Karnataka, India). HimLay® mainly comprises of *L. usitatissimum, A. racemosus, R. sativus, L. reticulata, T. cordifolia,* and *L. sativum.*

B. Experimental Diet Preparation

The experimental diet was formulated based on the prevailing nutritional standards for WL BV 300 egg-laying chicken (Kavi Protein and Feed Pvt. Ltd., Bengaluru, India) (Table I).

Table I Ingredients and nutrient composition of WL BC 300 layer basal diet

Ingredients	Quantity (g/kg)
Maize	644.800
Soybean meal	216.600
Vegetable oil	3.390
DiCalcium phosphate	11.680
Stone grit	115.600
Salt	4.138
DL-methionine	1.393
Trace mineral premix	1.000
Vitamin premix	0.500
Choline chloride	0.500
Nutrient composition	
ME, kcal/kg	2650.000
Crude protein, %	15.500
D-lysine	0.710
D-TSAA	0.600
D-tryptophan	0.166
D-threonine	0.553
D-isoleucine	0.592
D-valine	0.664
Calcium, %	3.800
NPP, %	0.300
Na	0.170

ME-metabolizable energy; Na-sodium; NPP-non-phytate phosphorus

The HimLay® was mixed in the basal diet at a rate of 500 g/ton.

C. General Layer Birds Husbandry Practices

The standard management practices required for WL BV 300 layer birds were followed during the course of the experiment. The experimental birds were reared in 4-bird colony cages (18″ × 12″ × 15″) using the stair-step cage system. Each cage had access to two fixed nipple drinkers and feeders. The potable drinking water and experimental diets were provided to the birds *ad libitum* throughout the study period. The recommended standard WL BV 300 layer bird's vaccination schedule was also followed.

D. Ethical Approval

This study was conducted according to the guidelines laid down for the care and use of animals, and the study protocol was approved by the Institutional Animal Ethics Committee, The Himalaya Drug Company, Bangalore, Protocol No. AHP/P/09/17. Institutional Ethics Committee.

E. Study Design

A total of 20 WL BV 300 nonlayer birds (diagnosed based on pelvic bone width) aged 28 weeks were procured from a local layer farmer and were allocated equally into two groups *viz* G1 and G2 (10 birds/group). G1 and G2 served as normal control and treatment groups, respectively, raised on normal commercial layer mash feed, and G2 was concurrently supplemented with HimLay® at a recommended dose of 500 g/ton for 8 weeks.

F. Assessment Parameters

Hen day egg production (HDEP) and pelvic bone width parameters were assessed weekly. Serum LH levels were estimated on day 0, week 4, and week 8. Ovarian activity was assessed at the termination of study.

G. HDEP

HDEP is usually expressed in percentage. It is mostly used for scientific studies and truly reflects the production capacity of the available birds in the house. The average for the whole egg-laying period of hen-day percent lay was determined and was termed as percent lay. Hen-day egg production per day was calculated by adopting the following formula given by North [10]. Hen-day egg production for the whole period was determined by summing up the daily hen-day egg production of the flock.

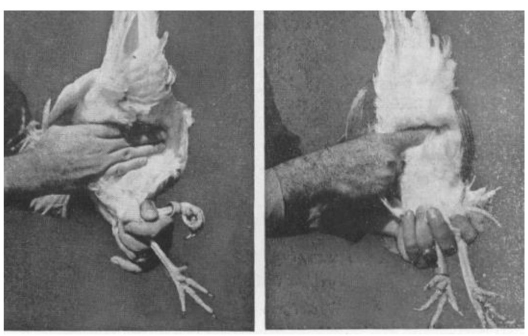
HDEP% = (Number of eggs produced daily/Number of birds available in the flock on that day) × 100

H. Serum LH Levels

Serum LH levels were measured using RIA according to the modified method of Bacon and Long [11].

I. Pelvic Bone Width Assessment

The hen was removed from the catching crate with as little disturbance as possible. Handheld with the palm facing upward, the hen was taken with her head under the arm, the forefinger between the legs, the thumb and the middle finger grasping the outside of the legs, and the fowl's body resting on the wrist and forearm. In this position, the bird can be easily handled, and every part of her body may be seen. The pelvic bone width finger scoring was assessed as demonstrated in Figure I.



Source: McCarthy 1938

Fig. I Assessment of pelvic bone width

J. Assessment of Ovarian and Follicle Activity

At the end of the study (35 weeks of age), two birds from each group were slaughtered by cervical dislocation, eviscerated, and the pelvic cavity was cut open. The reproductive organs *viz* ovary, uterus, and follicle distribution *viz* large yellow follicle, small yellow follicle, large white follicle, and small white follicle and activity were assessed, and findings were recorded.

III. RESULTS

The results of the pelvic bone width depicted that the conversion (%) of nonlayer birds to layer birds was increased during the treatment period of HimLay®. The nonlayer to layer bird conversion (%) on week 28, 29, 30, 31, 32, 33, 34, and 35 was 0%, 10%, 20%, 40%, 60%, 70%, 70%, and 80%, respectively (Table II).

Table II Effect of himlay® on the pelvic bone width in WL BV 300 non-layer birds

Age in weeks	G1-normal control			G2-HimLay® (500 g/ton)			Non-layer to layer conversion
	S	D	T	S	D	T	(%)
28	2	0	0	5	5	0	0
29	1	0	0	5	4	1	10
30	1	0	0	5	3	2	20
31	1	0	0	1	5	4	40
32	1	0	0	0	4	6	60
33	1	0	0	0	3	7	70
34	1	0	0	0	3	7	70
35	1	0	0	0	2	8	80
S-single finger; D-double finger; T-three fingers							

The egg production (%) in G2 on week 28, 29, 30, 31, 32, 33, 34, and 35 was 13%, 18%, 21%, 29%, 48%, 73%, 76%, and 86%, respectively (Table III).

Table III Effect of himlay® on egg production performance in WL BV 300 layer birds

Age in weeks	G1-normal control	G2-HimLay® (500 g/ton)	BV 300 standards (Std.)	Difference (G2-Std.)
28	0.00	13.00	97.00	−84.00
29	0.00	18.00	97.00	−79.00
30	0.00	21.00	97.00	−76.00
31	0.00	29.00	97.00	−68.00
32	0.00	48.00	96.90	−48.90
33	0.00	73.00	96.80	−23.80
34	0.00	76.00	96.60	−20.60
35	0.00	86.00	96.40	−10.40

The results of egg production data depicted that egg production was initiated from nonlayer birds as early as from the first week of HimLay® treatment. The egg production in G2 was increased throughout the treatment period, i.e., week 28–week 35. Furthermore, the gap difference of the actual egg production in G2 as compared with BV 300 layer standard egg production was reduced during the treatment period with HimLay.

HimLay® supplementation improves serum LH levels in G2 compared with G1 since a higher LH level of >0.1 mIU/ml was observed in the G2 group (Figure II).

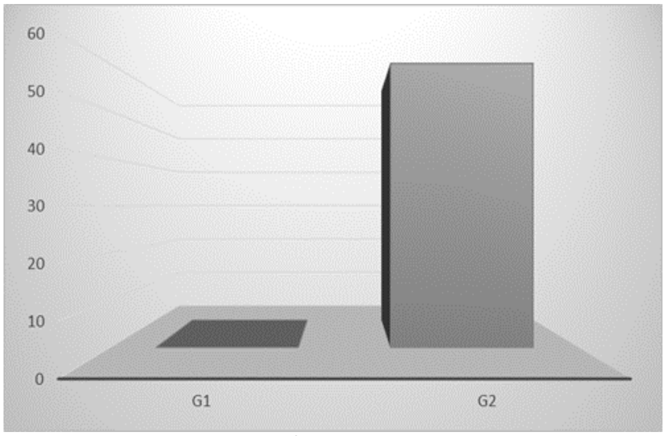


Fig. II Effect of HimLay® on serum LH levels in WL BV 300 layer birds

Functioning ovary and a hierarchy of developing follicles (yolks) were observed in layer birds in the G2 group between four and six large yolk-filled follicles. The largest one is the next to be ovulated to produce an egg (Table IV and Figure III).

Table IV Effect of himlay® on follicle distribution in WL BV 300 layer birds

Groups	LYF	SYF	LWF	SWF
G1-normal control	0	0	0	0
G2-HimLay® (500 g/ton)	5.80	6.00	14.40	>30.00
LYF-large yellow follicle; SYF-small yellow follicle; LWF-large white Follicle; SWF-small white follicle				

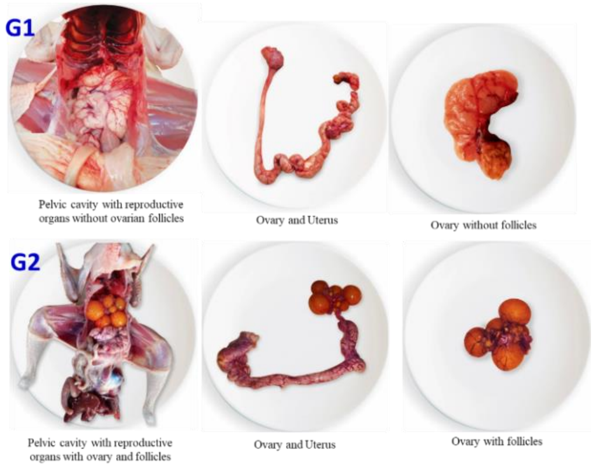


Fig. III Effect of HimLay® on follicle distribution in WL BV 300 layer birds

IV. DISCUSSION

Profits from the average farm poultry flock could be increased if owners would study the birds and learn to distinguish good layers, poor layers, and nonlayers. To ensure the efficiency and profitability of livestock production and to sustain food security due to the increasing demand of livestock produce, an enhancement regarding nutrition together with other management practices *viz* indentification layers and nonlayers is crucial for the maintenance of animal health, increasing production performance, and thus making poultry business profitable to poultry farmers. In the layer poultry industry, the manipulation of feed compositions, including introducing phytogenic feed additives in layer diets, has been studied to improve the egg production performance. Furthermore, studies also showed that phytogenics have the potential to be natural antibiotics as an alternative to the antibiotic cum growth promoters, which have been banned since 2006. Hence, the present study was designed and was conducted to evaluate the effect of HimLay® on egg lay, egg production, ovarian activity, and serum LH levels in WL BV 300 nonlayer birds.

Every person engaged in poultry production, regardless of the size of the enterprise, should have a working knowledge of culling. The owner of the flock should decide on culling of the flock based on the efficient use of feed by the hen in the manufacture of eggs but not upon someone else's opinion. The objective of culling is to remove from the flock those hens that do not make efficient use of their feed. Profits from the average farm poultry flock could be increased if owners would study the birds and learn to distinguish the good layers from the poor ones. Among many different methods adopted by the farmers to distinguish flocks between those that are nonlaying and laying and good layer and poor layer, pelvic bone width finger scoring is another one of the most important methods to distinguish the flocks since in a good layer, the pelvic bones are spread wide apart and become soft, thin, and pliable.

The distance between the pelvic bones and the keel bone also increases due to the increased size and length of the intestines and the enlargement of the reproductive organs. Hence, pelvic bone width measuring 1–2 and 3–5 fingers are distinguished as nonlayers and layers, respectively [1]. In our study, results of pelvic bone width depicted that the conversion (%) of nonlayer birds to layer birds was increased during the treatment period of HimLay®, and by the end of the 8th week treatment, 80% of the nonlayer hens were converted to layers.

The findings of egg production data described that egg production was initiated as early as from the first week of HimLay® supplementation, and egg production was increased gradually throughout the treatment period. Furthermore, almost 80% of BV 300 nonlayer birds supplemented with 500-g/ton HimLay® were able to produce eggs at par with BV 300 layer standards on week 35. These findings could be attributed to the positive effects of phytoactives present in the herbal ingredients of HimLay®. Literature reports demonstrated that phytoactives could exert augmentation of hepatocyte function in the liver (metabolism) and reproductive tracts (estrogen) by improving the synthesis of vitellogenin, which then stimulates the deposition of egg yolk in the developing follicles, and thus resulting in increased egg production [12,13]. Moreover, the findings of egg production in our study are in accordance with the previous findings reported by Akdeimar et al. [14], Nadia et al. [15], Park et al. [16], Damaziak et al. [17], and Abou-Elkhair et al. [18] wherein the addition of the different phytogenic feed additives in the layer bird's diet significantly increased the hen-day egg production. These findings could be credited to the effect of phenolic compounds from phytogenic feed additives, which helps in the digestion and absorption of the nutrients from the gastrointestinal tract by increasing enzymatic activity [19].

The initiation of production performance of WL BV 300 layer birds following supplementation of 500-g/ton HimLay® in our study could be understood from the improved serum LH levels, initiation of ovarian functions, and development of follicles. The literature has revealed that subcutaneous injection of FSH into old hens caused an increase in estrogen concentrations, increased the number of small follicles in their ovaries, and then stimulated the rapid growth phase of small follicles in layer birds [20]. Isoflavones is a major phyto-estrogen that exists widely in natural plants [21]. Some of the herbs in our feed additive mixture have estrogenic properties. Hence, in this study, the weight of the ovary and the number of small follicles were positively influenced following 500-g/ton HimLay® supplementation. Furthermore, serum LH levels were improved by adding polyherbal formulation of HimLay® in the diets of WL BV 300 nonlayer birds. According to Amirshekari *et al.*, FSH and LH lead to an increased diameter of mature follicles, an effect that may also explain the increase in ovarian weight [22].

V. CONCLUSIONS

In conclusion, to the best of our literature knowledge, the preliminary findings of this study demonstrated that supplementation of 500-g/ton HimLay® helps to increase serum estrogen hormones, initiation of ovarian functions, and development of follicles and thereby initiate egg production from WL BV 300 nonlaying hens. Thus, HimLay® helps in the conversion of nonlayer birds to layer birds, and hence, usage of HimLay® could play a crucial role in the profit of average poultry farmers by avoiding the unnecessary culling of nonlaying hens.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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