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Effect of Inclusion of Hatchery Discarded Infertile Egg Meal with Shell on Growth Performance, Nutrients Digestibility, Blood-Biochemical Profile and Immune Status of Labrador Puppies

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ABSTRACT

We conducted this study to examine how including infertile egg meal with shell obtained from the hatchery affects the growth, digestibility of nutrients, and health status of Labrador puppies. On the basis of the results of *in vitro* digestibility, two best-performing levels (2.5% IEMS and 5% IEMS diets) were selected from an *in vivo* study on Labrador puppies for their potential as an alternate protein source. For the experimental duration of 90 days, Group I fed a commercial puppy food (control)-based diet; Group-II fed a 2.5% IEMS-based diet; and Group-III fed a 5% IEMS-based diet. The results of the *in vivo* experiment revealed that there was no significant ($p > 0.05$) difference in final body weight, total weight gain, ADG, or FCR among all the treatment groups. The percent digestibility of DM, OM, and CP was significantly ($p < 0.05$) higher in the commercial puppy food and 2.5% IEMS-based diet than in the 5% IEMS diet; however, the commercial puppy food and 2.5% IEMS diet are comparable ($p > 0.05$) to each other. The percent digestibility of EE was significantly ($p < 0.05$) higher in the 2.5% IEMS and 5% IEMS diets as compared to the commercial puppy food groups. The blood biochemical parameters of Labrador puppies were assessed at the 0^{th}, 45^{th}, and 90^{th} days of the experiment. All the observed blood biochemical parameters were found to be comparable ($p > 0.05$) among the dietary treatments at the 0^{th} day, 45^{th} day, and 90^{th} day. The cell-mediated immune response to intradermal injection of PHA-P at the end of the experimental duration revealed that there was no significant ($p > 0.05$) difference in cell-mediated immune response among the different treatment groups. It may be concluded from the present study that 2.5% of IEMS can be incorporated in the diet of Labrador puppies as an alternate source of protein.

HIGHLIGHTS

- Inclusion of 2.5% and 5% IEMS in diets had no adverse effect on growth performance, feed efficiency, or health status.
- Nutrient digestibility of dry matter, organic matter, and crude protein was highest in the control and 2.5% IEMS diets, while ether extract digestibility improved with IEMS inclusion.
- Blood biochemical and immune response parameters remained comparable across all dietary treatments, indicating the safety of IEMS inclusion up to 2.5%.

Keywords: Infertile egg meal, hatchery waste, Labrador puppies, nutrient digestibility, alternative protein source

As the poultry industry is growing rapidly large amount of poultry waste is being produced in the forms solid and liquid mainly from hatchery, poultry farms and processing plants. Typically poultry hatchery produces solid and liquid waste, the solid waste comprises of the residue that left after the incubation process is completed and

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mainly classified into shells, infertile eggs, dead-in-shell and decaying tissue (Sung and Kim, 2020). During the years 2018–19, hatcheries in India produced an average of 9486 million metric tons of waste (Prabakaran and Valavan, 2021). The conventional method of disposing of solid hatchery waste is land-filling and spreading it on the pasture, resulting in soil and water pollution. Therefore, a sustainable, efficient, effective, and environmentally friendly method of disposal of hatchery waste is the need of the hour. One such alternate can be the incorporation of hatchery waste in the diet of animals, which not only solves the problem of environmental pollution but also makes a quality feed ingredient available for the feeding of the animals. Rejected infertile eggs during the candling process are considered a good source of high-quality protein, vitamins, and minerals, as well as competitiveness, compared to the prices of conventional protein feed ingredients like soybean and fish meal. So, it has gained increasing attention as a potential ingredient in animal diets. The pet population in India grew significantly from 18.1 million pets in 2016 to 31.7 million pets in 2021, with a compound annual growth rate (CAGR) of 11.9%, and is projected to reach 50.1 million pets by 2026 with a CAGR of 9.6%. This encourages the growth of the pet food industries in India, and the sale of pet food increased significantly, achieving USD 403.4 million in 2021 with a CAGR of 18.5% and expected to attain a valuation of USD 887.6 million by 2026 with a CAGR of 17.1%. (Bernardi, 2023). Due to this demand, there is a search for alternative protein sources that can be included in pets' diets. So, infertile eggs from the 7^{th} or 18^{th} day of the candling process in the hatchery during the incubation process can be a good source of alternative protein in the diet of dogs, so, considering these above-mentioned points, the existing study is planned with the objective of investigating the effect of the inclusion of hatchery-discarded infertile egg meal with shell on the growth performance, nutrient digestibility, blood-biochemical profile, and immune status of Labrador puppies.

MATERIALS AND METHODS

Housing and environment

The puppies were housed in a well-ventilated kennel facility. Each puppy was individually accommodated in a cage measuring 5 feet in length, 3 feet in width, and 4 feet in height. The kennel environment was maintained under controlled conditions to ensure animal welfare, with the temperature set between 18°C and 24°C, a 12-hour light/dark cycle for illumination, and humidity regulated between 37% and 70%. To maintain hygiene, the facility and individual enclosures were cleaned twice daily. To help the puppies adapt to the study environment and familiarize them with handling, they received regular interaction with caretakers and researchers. The health status of each puppy including physical appearance, body weight progression, coat quality, and any signs of illness were carefully monitored throughout the trial. For identification, each puppy wore a collar with a metallic tag corresponding to its assigned treatment group.

Dietary treatments

The two best performed level of infertile egg meal with shell diet (2.5% and 5%) was selected based on the *in vitro* digestibility study Group I was fed with a commercial puppy feed, group-II was fed with a 2.5% infertile egg meal with shell (IEMS) based diet and group-III was fed with 5% infertile egg meal with shell based diets as per AAFCO (2014) and energy density of diet will be kept 3500 Kcal ME/kg in accordance with the Indian climatic conditions as described by ICAR (2013) for the experimental duration of 12 weeks.

Growth performance and nutrient digestibility

The growth performance of the puppies was evaluated by recording their body weights every two weeks using an electronic weighing scale. Weighing was done in the morning, prior to feeding and watering, and continued consistently over the 12-week study period. Any increase in body weight from the initial measurement was considered as growth or weight gain. To assess nutrient digestibility, a four-day digestion trial was carried out at the conclusion of the experiment. Daily, after recording the total quantities, representative samples of the feed offered, feed refusals, and feces were collected on an individual animal basis and preserved for laboratory analysis. The pooled, dried samples of feed, leftovers, and feces were analyzed for proximate composition following the methods described by AOAC (2005). Nutrient digestibility was then computed using standard calculation methods.

% digestibility =

$$\frac{\text{Amount of nutrient consumed} - \text{Amount of nutrient excreted in faeces}}{\text{Amount of nutrient consumed}} \times 100$$

Palatability score

Utilizing the subjective assessment procedure recommended by Strickling *et al.* (2000), a 4-point scale was used to score the palatability of experimental pet foods and was assessed during the digestion trial.

Blood-biochemical profile

Blood samples (4 ml) were collected from each puppy on days 0, 45, and 90 of the feeding trial. A portion of the sample was placed in EDTA vials for hematological analysis, while the remainder was collected in serum activator tubes for serum biochemical evaluation. The serum was analyzed for various biochemical parameters and mineral profiles using standard diagnostic kits with an automated clinical chemistry analyzer (Vitros® 350 Chemistry System, Ortho-Clinical Diagnostics Inc., Johnson & Johnson, SA).

Cell-Mediated Immune Response

To assess the *in vivo* cell-mediated immune function, a type-IV delayed-type hypersensitivity (DTH) reaction was induced by intradermal administration of phytohaemagglutinin-P (PHA-P) mitogen (0.5 mg/ml in saline) at the end of the feeding trial. The injection site was clipped and cleaned with 70% ethanol prior to administering 100 µl of the solution. The DTH response was monitored by measuring skin thickness at 0, 12, 24, 48, and 72 hours post-injection using a digital Vernier caliper. The immune response was quantified as the percentage increase in skin thickness relative to the baseline measurement (Kumar *et al.*, 2017).

Statistical analysis

The data generated in the above experiments were statistically analyzed using IBM SPSS version 20 computer package. For comparison of groups, generalized linear model ANOVA procedure and Duncan's multiple range test were used (Snedecor & Cochern, 1994). Significant difference among the treatments was established at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of the diet

The data pertaining to the chemical compositions of extruded diets prepared from graded level (2.5 % and 5%) of inclusion of infertile egg meal with shell (IEMS) and commercial puppy food is presented in Table 1.

Table 1: Chemical composition of extruded puppy food prepared from graded level of infertile egg meal with shell (IEMS) (on DM basis)

Parameters (%)	CPF*	2.5% IEMS	5% IEMS
Dry Matter	91.20	90.30	90.70
Crude Protein	25.38	23.63	23.41
Ether Extract	7.35	9.65	9.58
Crude Fibre	3.49	3.28	3.78
Calcium	1.10	1.42	1.36
Phosphorus	1.07	1.12	1.10
Total Ash	7.93	5.47	5.73
Acid Insoluble Ash	0.47	0.39	0.52

*Commercial puppy food. #IEMS: infertile egg meal with shell.

The results revealed that the DM, CP, EE, CF, TA, AIA, Ca and P was 91.20 %, 25.38 %, 7.35%, 3.49%, 7.93%, 0.47%, 1.1% and 1.07% respectively, in commercial puppy food. The extruded puppy diet prepared from inclusion of 2.5% infertile egg meal with shell (IEMS) on chemical analysis revealed that the DM, CP, EE, CF, TA, AIA, Ca and P was 90.30%, 23.63%, 9.65%, 3.28%, 5.47%, 0.39%, 1.42% and 1.12% respectively. Chemical analysis of extruded diets prepared from 5% level of inclusion of infertile egg meal with shell (IEMS) had 90.70% 23.41%, 9.58%, 3.78%, 5.73%, 0.52%, 1.36%, and 1.10%, DM, CP, EE, CF, TA, AIA, Ca and P respectively. Which indicates that the diet fed to puppies in the different treatment groups was adequate to support normal growth as per AFCO (2014).

Growth performance

The data pertaining to growth performance parameters of Labrador puppies viz. initial body weight, feed conversion ratio (FCR), final body weight, total weight gain, average daily gain (ADG), and daily dry matter intake (DMI) is presented in Table 2.

Table 2: Effect of different levels of infertile egg meal with shell on growth performance of Labrador puppies

Attributes	Groups			SEM	P value
	CPF	2.5% IEMS	5% IEMS		
Initial body weight (kg)	18.34	18.07	18.36	1.722	0.998
Final body weight (kg)	26.90	26.52	26.68	1.334	0.995
Total body weight gain (kg)	8.56	8.45	8.32	0.399	0.978
ADG (g)	95.11	93.85	92.44	4.435	0.978
DMI (g)	409.99	406.35	408.10	0.531	0.968
FCR	20.88	20.79	20.39	0.979	0.982

The result revealed that the initial body weight, final body weight, average daily gain (ADG), feed conversion ratio (FCR) and daily dry matter intake (DMI) was non-significant ($p > 0.05$) among all the treatment groups. Similarly, Pandurang (2022) reported that final body weight, body weight gain, FCR was not affected ($p > 0.05$) upto 2.5% level of inclusion of whole infertile egg meal in broiler chicken for 42 days however, it was observed when the level of whole infertile egg meal was increased upto 10%, final body weight and body weight gain increases significantly ($p < 0.05$). Contrary, to our observation Akinola *et al.* (2020) reported significantly ($p < 0.05$) lower final body weight and body weight gain in broiler chicken when the level of infertile egg meal with shell increases gradually (0%, 20%, 60% and 100%) however, the FCR remains comparable ($p > 0.05$) among all the treatment groups. The results of the present study varied with the results of the studies conducted so far may be due to difference in the levels of inclusion of infertile egg meal with shell (IEMS) and duration of the experiment.

Digestibility of the nutrients

The data pertaining to percent dry matter digestibility, crude protein digestibility, ether extract digestibility and crude fibre digestibility of Labrador puppies fed different dietary treatment is presented in table 3. The dry Matter digestibility of commercial puppy food (85.82%) and 2.5% IEMS (82.95%) diet was significantly ($p<0.05$) higher than 5% IEMS (78.57%) diet however, Dry Matter digestibility of commercial puppy food (85.82%) and 2.5% IEMS (82.95%) was found to comparable ($p > 0.05$) with each other. The organic Matter digestibility of commercial puppy food (87.91%) and 2.5% IEMS (86.77%) diet was significantly higher than 5% IEMS (83.28%) diet ($p < 0.05$) however, organic Matter digestibility of commercial puppy food (87.91%) and 2.5% IEMS (83.28%) was comparable was found to comparable ($p > 0.05$) with each other. The crude protein digestibility of commercial puppy food (83.20%) and 2.5% IEMS (83.27%) diet was significantly ($p < 0.05$) higher than 5% IEMS (79.14%) diet ($p < 0.05$) however, crude protein digestibility of commercial puppy food (83.20%) and 2.5% IEMS (83.27%) was comparable was found to comparable ($p > 0.05$) with each other.

Table 3: Digestibility of the nutrients in Labrador puppies fed different level of infertile egg meal with shell (IEMS)

Attributes	Groups			SEM	p-value
	CPF	2.5% IEMS	5% IEMS		
Dry Matter digestibility (%)	85.82^{a}	82.95^{a}	78.57^{b}	1.165	0.006
Organic matter digestibility (%)	87.91^{a}	86.77^{a}	83.28^{b}	0.781	0.009
Crude protein digestibility (%)	83.20^{a}	83.27^{a}	79.14^{b}	0.811	0.025
Ether extract digestibility (%)	92.78^{a}	95.78^{b}	95.46^{b}	0.731	0.024
Crude fibre digestibility (%)	51.36	51.66	49.98	2.393	0.965

^{ab}mean bearing different superscript within a column differ significantly ($p<0.05$).

The ether extract digestibility was significantly ($p < 0.05$) higher in 2.5% IEMS (95.78%) diet and 5% IEMS (95.46%) diet than the commercial puppy food (92.78%) diet. Crude fibre digestibility of commercial puppy food, 2.5% IEMS and 5% IEMS was 51.36%, 54.66% and 49.98 % respectively, and was comparable among all the treatment groups. These findings of nutrients digestibility was in close agreement with the study conducted by Pandurang *et al.* (2022) who reported that digestibility of

DM, OM, EE and CF were remain comparable ($p > 0.05$) in broiler fed graded level of whole infertile egg meal (0%, 2.5%, 5, 7.5% and 10 %) for the experimental duration of 42 days. However, digestibility of crude protein was significantly ($p < 0.05$) higher when the level of whole infertile egg meal was increased.

Haematological parameters

The data of the blood parameters of Labrador puppies of fed CPF, 2.5% IEMS and 5% IEMS diets taken at beginning (0 day), mid (45 days), and end (90 days) of the experiment is presented in table 4, 5 and 6.

Table 4: Complete blood count of Labrador puppies in different groups at 0 day

Parameter	Treatment				
	CPF	2.5% IEMS	5% IEMS	SEM	p value
Hb (g/dL)	13.70	13.50	13.93	0.330	0.896
TEC ($\times 10^6$) (per cu mm)	5.63	5.89	5.67	0.059	0.155
PCV %	38.60	39.57	38.40	0.357	0.415
PLT ($\times 10^3$) (per cu mm)	291.33	238.67	257.67	14.514	0.371
TLC ($\times 10^3$) (per cu mm)	15.23	14.53	13.57	0.836	0.770

Table 5: Complete blood count of Labrador puppies in different groups at 45 day

Parameter	Treatment				
	CPF	2.5% IEMS	5% IEMS	SEM	p value
Hb (g/dL)	14.80	12.60	14.00	0.587	0.343
TEC ($\times 10^6$) (per cu mm)	5.92	5.84	5.78	0.108	0.892
PCV %	41.93	39.67	40.63	0.709	0.485
PLT ($\times 10^3$) (per cu mm)	352.00	256.33	260.33	25.569	0.246
TLC ($\times 10^3$) (per cu mm)	14.90	16.63	16.00	0.802	0.730

All of the observed blood parameters pertaining to Hb, TEC, PCV, PLT and TLC were found to be comparable ($p > 0.05$) among the dietary treatment at 0 day, 45 days and 90 days values lie within the reference range confirming that puppies were healthy. Similarly, Akinola *et al.* (2020) reported that no significant ($p > 0.05$) difference was observed in the blood parameters viz. haemoglobin, PCV and RBC after feeding different forms of the infertile eggs (IEM and IEMS) in broiler. Also results were (Lie and Kim. 2013) reported no significant ($p > 0.05$) effect on the RBC and lymphocytes count in broiler fed whole egg powder at the rate of 1%, 2% and 3%.

Table 6: Complete blood count of Labrador puppies in different groups at 90 day

Parameter	Treatment				
	CPF	2.5% IEMS	5% IEMS	SEM	P value
Hb (g/dL)	13.87	12.73	13.90	0.619	0.739
TEC ($\times 10^6$) (per cu mm)	5.94	5.84	5.90	0.167	0.974
PCV %	39.00	39.57	40.77	1.258	0.877
PLT ($\times 10^3$) (per cu mm)	261.33	257.67	263.33	4.129	0.884
TLC ($\times 10^3$) (per cu mm)	13.87	15.93	16.10	0.966	0.640

Serum biochemical parameters

The data of the serum biochemical parameters of Labrador puppies fed different diets, examined at 0, 45 and 90 days of experimental feeding is presented in table 7, 8 and 9.

Table 7: Serum biochemical parameters of Labrador puppies fed different level of infertile egg meal with shell at 0 day

Parameter	Treatment				
	CPF	2.5% IEMS	5% IEMS	SEM	P value
Na^+ (mEq/L)	132.33	162.67	136.33	7.461	0.209
K^+ (mEq/L)	4.70	4.80	4.90	0.309	0.974
Cl^- (mEq/L)	92.33	91.33	107.33	6.135	0.556
Ca^{++} (mg/dl)	9.00	8.93	10.33	0.601	0.627
P_i (mg/dl)	5.87	6.23	6.50	0.580	0.927
GLU (mg/dl)	94.00	89.67	111.67	6.807	0.432
TP (g/dl)	6.03	6.10	6.13	0.148	0.971
ALB (g/dl)	2.90	2.90	2.87	0.086	0.987
Triglycride (mg/dl)	31.67	45.67	51.00	6.570	0.527

Cholesterol (mg/dl)	170.00	172.33	177.33	11.524	0.974
BUN (mg/dl)	13.33	15.00	13.33	0.857	0.717
Creatinine (mg/dl)	0.83	0.83	0.97	0.060	0.638
AST (U/L)	31.00	35.00	44.33	3.700	0.366
ALT (U/L)	28.33	30.67	38.00	2.261	0.200
AKP (U/L)	91.67	84.00	116.67	15.369	0.722

Table 8: Serum biochemical parameters of Labrador puppies fed different level of infertile egg meal with shell at 45 day

Parameter	Treatment				
	CPF	2.5% IEMS	5% IEMS	SEM	P value
Na^+ (mEq/L)	134.67	127.67	135.33	5.757	0.870
K^+ (mEq/L)	93.33	99.33	104.00	4.806	0.721
Cl^- (mEq/L)	4.70	4.80	4.90	0.309	0.974
Ca^{++} (mg/dl)	8.83	10.03	10.77	0.571	0.432
P_i (mg/dl)	6.73	6.53	6.90	0.557	0.973
GLU (mg/dl)	98.33	100.67	113.33	4.837	0.453
TP (g/dl)	6.10	6.47	6.20	0.173	0.730
ALB (g/dl)	3.10	2.87	2.90	0.128	0.777
Triglycride (mg/dl)	52.33	52.33	46.00	3.370	0.734
Cholesterol (mg/dl)	227.67	172.33	172.67	15.671	0.282
BUN (mg/dl)	12.33	18.33	15.67	1.519	0.306
Creatinine (mg/dl)	0.83	0.97	1.10	0.071	0.348
AST (U/L)	26.00	34.00	39.67	3.876	0.403
ALT (U/L)	29.00	28.67	37.00	3.145	0.536
AKP (U/L)	74.00	94.33	118.33	14.123	0.501

Table 9: Serum biochemical parameters of Labrador puppies fed different level of infertile egg meal with shell at 90 day

Parameter	Treatment				
	CPF	2.5% IEMS	5% IEMS	SEM	P value
Na^+ (mEq/L)	130.00	126.33	153.00	7.844	0.369
K^+ (mEq/L)	102.00	104.33	102.67	4.833	0.985
Cl^- (mEq/L)	5.10	4.80	5.53	0.247	0.539
Ca^{++} (mg/dl)	10.17	9.83	11.70	0.629	0.496
P_i (mg/dl)	6.53	6.10	6.70	0.371	0.836
GLU (mg/dl)	96.00	93.33	97.67	5.091	0.955
TP (g/dl)	6.03	7.17	7.60	0.393	0.271
ALB (g/dl)	3.00	2.83	3.13	0.095	0.495
Triglycride (mg/dl)	56.67	46.00	50.67	2.679	0.298
Cholesterol (mg/dl)	161.67	158.67	172.00	6.861	0.762
BUN (mg/dl)	17.33	24.00	24.00	1.544	0.112
Creatinine (mg/dl)	1.03	0.87	1.27	0.091	0.214
AST (U/L)	29.33	34.00	36.33	4.523	0.339
ALT (U/L)	37.67	36.67	39.33	2.508	0.930
AKP (U/L)	70.67	98.67	97.00	8.125	0.327

There is no significant difference ($p > 0.05$) between the serum biochemistry among the different dietary groups on 0, 45 and 90 days of experimental feeding and the values lies within the normal reference range. Similarly, Akinola *et al.* (2020) reported that no significant difference was observed in the blood biochemical parameter viz. albumin and Creatinine after different forms of the infertile eggs (IEM and IEMS) in broiler however, there were significant ($p < 0.05$) higher total protein level in the serum with increase in the level of infertile egg meal. Similarly, Pandurang *et al.* (2022) reported no significant difference blood biochemical profile of broiler fed graded level of whole infertile eggs meal for 42 days. Contrary to our observation Esmailzadeh *et al.* (2012) reported higher serum total protein, cholesterol and triglyceride level as the level of egg powder increases in the diet of the broilers.

Antioxidant status

The data pertaining to antioxdant status revelaed that the activity. Superoxide dismutase (SOD) Group I with CPF food, Group II with 2.5% IEMS diet, and Group III with 5% IEMS diet was 118.05 U/mgHb, 117.89 U/mgHb, and 108.69 U/mg Hb respectively. Statistical analysis revealed that there was no significant ($p > 0.05$) difference in superoxide dismutase (SOD) activity among different groups catalase activity of Group I with CPF food, Group II with 2.5% IEMS diet, and Group III with 5% IEMS diet was 86.23 U/mgHb, 82.49 U/mg Hb, and 80.89 U/mg Hb respectively. Statistical analysis revealed that there was no significant ($p > 0.05$) difference in catalase activity among different groups. Similarly, Pandurang *et al.* (2022) reported no significant ($p > 0.05$) in the activity of SOD and catalase activity in broiler fed graded level of whole egg powder for 42 days.

Table 10: Cell mediated immune response of Labrador puppies

Groups	Hours post injection						Mean	SEM	p-Value		
	0	12	24	48	72	96			G	p	g*p
CPF	4.57	7.04	6.92	6.22	5.50	4.893	5.858	0.636	0.135	0.001	0.871
2.5 % IEMS	5.67	8.45	7.00	6.48	6.00	5.47	6.511				
5 % IEMS	5.13	6.09	6.8	6.33	5.55	5.17	5.855				
Mean	5.123[a]	7.20[c]	6.92[c]	6.34[bc]	5.69[ab]	5.18[a]					

[abc]Mean bearing different superscript within the column differ significantly (P<0.05).

Cell mediated immune response

The data of the Cell mediated immune response of Labrador puppies of Group I with CPF food, Group II with 2.5% IEMS diet, and Group III with 5% IEMS diets is presented in table 10. The Cell mediated immune response in terms of absolute increase in the skin indurations shoed the maximum values in all the three groups commercial puppy food, 2.5% IEMS diet and 5% IEMS diet at 12-hr post-inoculation of PHA-P, beyond which there was a steady decline till 96-h post-inoculation. The corresponding values for Group I with CPF food, Group II with 2.5% IEMS diet, and Group III with 5% IEMS diets were 5.85 mm, 6.51 mm and 5.85 mm respectively. There is no significant (p>0.05) difference between the three groups. Esmailzadeh *et al.* (2013) reported in research that an increase in the thickness of the broiler combs was used to measure the immune system's reaction to PHA injection. There was essentially no significant difference between broiler fed diets with 1, 2 and 3 % of egg powder (P>0.05). It may be concluded from the present study that 2.5 % of IEMS can be incorporated in the diet of Labrador puppies as an alternate source of protein.

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