Indian J. Anim. Nutr. 2022. 39 (4): 480-485
doi: 10.5958/2231-6744.2022.00054.8

Indian Journal of Animal Nutrition

Effect of Inclusion of Hatchery Discarded Infertile Egg Meal with Shell on *In Vitro* Digestibility of Puppy Food

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ABSTRACT

The present study was undertaken to investigate the effect of inclusion of infertile egg meal with shell obtained from the hatchery on *in vitro* digestibility of commercial puppy food. The infertile egg meal obtained from the GADVASU hatchery was first broken in a tray along with shell and dried in a hot air oven at 65°C for 48 hours and then grinded to form infertile egg meal with shell (IEMS). The IEMS was subsequently analysed for microbiological examination and *in vitro* digestibility of puppy food at graded inclusion level of 2.5, 5, 7.5 and 10%. The chemical analysis of the IEMS revealed that the DM, CP, EE, CF, TA, AIA, Ca and P was 35.5, 33.2, 26.5, 0.1, 20.5, 0.1, 9.1 and 1.13 %,respectively. The microbiological examination revealed that IEMS samples were negative for Salmonella and *E. coli* bacteria, while the total viable count was 2060 CFU at 1:10 dilution which subsequently become nil in further dilution and thus the bacterial contamination of IEMS was negligible. Results revealed that the *in vitro* digestibility of dry matter was significantly ($P<0.05$) highest in commercial puppy food (87.3 %) and 2.50 % level of IEMS (86.1%) and was significantly ($P<0.05$) lowest at 10% level of IEMS (82.94%). The *in vitro* organic matter digestibility was significantly ($P<0.05$) higher in commercial puppy food (88.4%) and 2.5% IEMS level (87.4%) as compared to 7.5 % (84.8%) and 10 % (84.0%) level of inclusion of IEMS however, it was comparable with 5% level of IEMS inclusion. The ether extract digestibility was significantly ($P<0.05$) highest in 2.5% level of IEMS (97.0%) and significantly ($P<0.05$) lowest in commercial puppy feed (91.9%). The crude protein digestibility was significantly ($P<0.05$) higher in the commercial puppy food, 2.5 %, 5 % and 7.5 % level of IEMS as compared to 10% level of IEMS. It was concluded that IEMS is a good source of alternative protein and can be included at 2.5 and 5 % level in the diet of Labrador puppy food.

KEYWORDS: Infertile egg meal with shell, *In vitro* digestibility, Microbiological examination Puppy food

Article received: 14 November 2022; Article accepted: 12 December 2022

INTRODUCTION

The enormous growth of poultry industry in past few decades leads to a large production of poultry by-products such as hatchery waste. Typically, hatchery waste contains the residue after the incubation process is completed and it can be classified into infertile eggs, unhatched eggs, dead chicks and hatched eggshells (Sung and Kim, 2020). In India, 9486 MT of hatchery waste was produced during 2018-19 (Prabakaran and Valavan, 2021). Currently, most of hatchery waste has been disposed by dumping in the landfill and spreading on the pasture. Environmental pollution in soil and water has increased due to application of large amount of hatchery waste to the soil. Therefore, it is necessary to establish an effective, environmentally friendly technique of disposal of hatchery waste. The infertile eggs are good source of crude protein, fat, calcium and essential amino acids of high biological value, as well as competitiveness, compared to the prices of soybean meal and fish meal. So, it has gained an increasing attention as a potential ingredient in animal diets. Pet food industries in India is growing rapid with CAGR of 13.9% and have a valuation of USD 297 million in 2019.This demand is creating a search for alternative protein sources that may be

included in diets of pets. Infertile eggs or egg with dead germ obtained from 7th or 18th day candling process in hatchery during incubation process can be a good source of alternative protein in the diet of dogs. Considering these facts, this study was planned with the objective to evaluate *in vitro* digestibility of Labrador puppy food with graded inclusion level of infertile egg meal with shell.

MATERIALS AND METHODS

Preparation of infertile egg meal with shell

Infertile eggs with shell were collected from the Guru Angad Dev Veterinary and Animal Sciences University hatchery on 7th or 18th day candling. After collection, they were broken along with the shells in a tray having dimensions of 81cm x42 cm x3.5 cmand then these trays carrying broken eggs (28-30 eggs per tray) were kept in a hot air oven for drying at 65°C for 48 hours and then grinded in a grinding machine to prepare infertile egg meal with shell (IEMS) and stored in a air tight container for further use.

Chemical evaluation of IEMS and the puppy diets

The IEMS and different puppy diets were analysed for proximate principles like dry matter, crude protein, ether extract, crude fibre, total ash and acid insoluble ash (AOAC, 2005). Calcium was estimated as per Talapatra et al. (1940) and available phosphorus as per the procedure described by AOAC (2005).

Microbiological analysis of IEMS

Salmonella

The procedure described in the OIE Terrestrial Manual (2008) and FDA Bacteriological Analytical Manual (2007)wereundertaken for the isolation and identification of Salmonella species. The IEMS sample processed for the isolation of Salmonella spp. after pre-enrichment, enrichment, and selective plating in the buffered peptone water, pre-enrichment was carried out by inoculating 1g of the IEMS in 9 ml of buffered peptone water and incubated for 24 hours at 37°C. Enrichment process was done by incubating 9.9 ml of Rappaport Vassiliadis (RV) medium with 100 ìl of the pre-enriched media at 42 °C for 18 to 24 hours.Selective plating procedure was followed by taking a loopful of the enhanced medium and streaked over the Xylose Lysine-Deoxycholate (XLD) agar plates and incubated at 37 °C for 24 hours. On the basis of cluster appearances, such as smooth, round, pin-pointed red colonies on XLD agar with or without black centres, probable identification was made.

Escherichia coli

The IEMS samples were processed for isolation of *E. coli* as per the method described by Lupindu (2017) with some modification. The egg samples were enriched for 24 hours at 37°C before being inoculated with germ-free Enterobacteriaceae Enrichment Broth (EEB). Selective plating was done by taking loop of enriched broth and straked over the Eosin Methylene Blue (EMB) agar and were incubated at 37°C plates for 24 hours. Based on cluster appearances, such as a metal-like shine on EMB agar, probable identification was made.

Total viable count

Total viable count is an estimate of number of microorganisms and the count is expressed as colony forming unit (CFU/ml). 1 g infertile egg meal with shell sample was added to 9 ml of 0.1% buffered peptone waterand diluted to 10^{-1}. Take 1ml from 10^{-1} tube with sterile pipette and transfer to another 9ml of 0.1% buffered peptone water making dilution 10^{-2}. Continue this process till dilution become $10^{-5}$and keep labelling them. Now label five normal agar plate as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Take 100 microlitre from respective buffered peptone watertube and add it to Normal agar plate with same label. With the help of spreader, spread all the inoculum on the surface of Normal agar. Repeat the process in every plate and incubate them at 37^0C for 24 h. For statistical reasons, use only data from plates which have between 30 and 300 colonies in this calculation. Each colony forming unit (CFU) represents a single cell or a group of cells attached together and inseparable by shaking. Therefore, the number of CFU in the original sample is determined by multiplying the number of colonies on a dilution

plate by the corresponding dilution factor.

In vitro evaluation of Labrador puppy diet containing infertile eggs meal with shell

The *in vitro* evaluation of puppy diet containing infertile eggs meal with shell at different levels (2.5, 5, 7.5 and 10 %) was done as per the method described by Biagi et al. (2016) consisting of two incubation phases i.e gastric phase of 2 hours in the presence of pepsin, gastric lipase and HCl and a second phase (intestinal phase) of 4 hours incubation in a buffer solution in the presence of pancreatin and bile salts. After that, undigested food samples was filtered using nylon bag and washed with cold water and kept in hot air oven for drying at 65^0C until constant weight is achieved. The dried samples were analysed for proximate principles to obtain the digestibility of the nutrients as per the method described by AOAC (2005).

Statistical analysis

The data generated was statistically analyzed using IBM SPSS (version 20) computer package. For comparison of groups, one way ANOVA procedure and Duncan's multiple range test was used (Snedecor and Cochran, 1994). Significance difference among the groups was established at $P<0.05$

RESULTS AND DISCUSSION

Chemical composition of infertile egg meal with shell (IEMS)

The chemical composition of infertile egg meal with shell (IEMS) prepared from 7^{th} or 18^{th} day candling process in hatchery during incubation process is presented in Table1. The result of chemical analysis of the IEMS revealed that the DM, CP, EE, CF, Ash, AIA, Ca and P was 35.5, 33.2, 26.5, 0.1, 20.5, 0.1, , 9.1 and 1.13 %, respectively. The results of chemical analysis of IEMS was in close agreement with the studies conducted by different researchers who reported 34.1-41.4 % CP, 18.0-27.6% total ash, 22.4-26.7 % EE, 0.59-3.76% CF, 0.4- 0.5 % P and 8.9-11.6% Ca in the infertile eggs or egg powder (Pandurang, 2022; Akinola et al., 2020; Choi et al., 2021; Ratriayanto et al., 2020).The variation in the chemical composition of infertile egg meal in these studies may be due to different time of collection of eggs from the hatchery or difference in the processing methods for preparation of infertile egg meal (Akinola et al., 2020).

Table 1. Chemical composition of infertile egg meal with shell (IEMS)

Parameters (%)	IEMS
DM	35.5
CP	33.2
EE	26.5
CF	0.1
Ca	9.1
P	1.13
TA	20.5
AIA	0.1

Chemical composition of different puppy food prepared with different levels of IEMS and commercial puppy food

Thedata pertaining to the chemical compositions of extruded diets prepared from graded level (2.5 %,5 %, 7.5 and 10%) of inclusion of infertile egg meal with shell (IEMS) and commercial puppy food is presented in Table 2. The results revealed that the DM, CP, EE, CF, TA, AIA, Ca and P was 91.2, 25.3, 7.35, 3.49, 7.93, 0.47, 1.10 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 90.3, 23.6, 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 IEMS had 90.7% DM 23.4% CP, 9.58% EE, 3.78%CF, 5.73% TA, 0.52% AIA, 1.36% Ca and 1.10% P. When chemical compositions of extruded diets prepared from 7.5% level of inclusion of IEMS was determined, the DM, CP, EE, CF, TA, AIA, Ca and P was 90.10, 23.19, 9.61, 3.90, 5.71,1.39 and

1.05%,respectively. Chemical analysis of extruded puppy diet prepared from 10% level of inclusion of IEMS had 90.80% dry matter, 23.19% crude protein, 9.47% ether extract, 4.05% crude fibre, 1.40% calcium, 1.01% phosphorus, 5.67% total ash, and 0.49% acid insoluble ash. It was revealed from the chemical composition of all the diets prepared from the graded level of inclusion of IEMS fulfil the nutrients requirements specified by AAFCO (2015) and ICAR (2013) for puppy food.

Table 2. Chemical compositions of extruded puppy feed diets prepared from graded level of inclusion of infertile egg meal with shell

Parameters (%)	Commercial Puppy Food	2.5% IEMS	5% IEMS	7.5% IEMS	10% IEMS
Dry Matter	91.2	90.3	90.7	90.1	90.8
Crude Protein	25.3	23.6	23.4	23.1	23.1
Ether Extract	7.35	9.65	9.58	9.61	9.47
Crude Fibre	3.49	3.28	3.78	3.90	4.05
Calcium	1.10	1.42	1.36	1.39	1.40
Phosphorus	1.07	1.12	1.10	1.05	1.01
Total Ash	7.93	5.47	5.73	5.71	5.67
Acid Insoluble Ash	0.47	0.39	0.52	0.52	0.49

DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; Ca: calcium; TA: total ash; AIA: acid in soluble ash; IEMS: infertile egg meal with shell; CPF: commercial puppy food.

Microbiological analysis of IEMS

The results pertaining to microbiological analysis of IEMS were presented in Table 3.

Table 3.Microbiology analysis of diets and IEMS

Treatments	*Salmonella*	*E. coli*	Total viable count (cfu)
IEMS	-ve	-ve	2060

IEMS: infertile egg meal with shell

Results revealed that it was negative for salmonella and *E. coli* bacteria, while the total viable count was 2060 CFU at 1:10 dilution which subsequently become nil in further dilution indicating that bacterial contamination of IEMS is very minimal or negligible. Similarly, Eldeek et al. (2005) reported the lower total viable count and E. *coli* count in hatchery discarded cooked and autoclaved eggs infertile egg meal. Pandurang (2022) also reported that hatchery discarded whole infertile egg meal was negative for Salmonella and *E. coli* count. So, it can be stated that from microbiological point of view infertile egg with shell obtained from hatchery were safe for the inclusion in the Labrador puppy diet.

In-vitro digestibility of puppy food with graded level of infertile egg meal with shell

The data of the*in vitro* digestibility experiment of puppy food with graded level of inclusion of IEMS after extrusion is presented in Table 4.

Table 4. *In vitro* digestibility of puppy food with graded level of infertile egg meal with shell

Parameters (%)	CPF*	2.50 % IEMS	5% IEMS	7.50% IEMS	10% IEMS	SEM	P value
Dry matter digestibility	87.30[a]	86.12[a]	85.28[ab]	83.71[bc]	82.94[c]	0.574	0.020
Organic matter digestibility	88.45[a]	87.44[a]	85.51[ab]	84.85[b]	84.05[b]	0.583	0.025
Ether extract digestibility	91.96[a]	97.07[b]	96.23[bc]	95.00[c]	94.96[c]	0.606	0.007
Crude protein digestibility	91.32[a]	92.08[a]	91.69[a]	90.96[a]	88.08[b]	0.514	0.027

[abc]Mean bearing different superscript within the column differ significantly (P<0.05); * Commercial puppy food [#]IEMS: infertile egg meal with shell

Results revealed that *in vitro* digestibility of dry matter was significantly (P<0.05) highest in commercialpuppy food (87.30 %) and 2.50 % level of IEMS (86.12 %) and was significantly (P<0.05) lowest at 10 % level of IEMS (82.94%). The *in vitro*organic matter digestibility was significantly (P<0.05) higher in commercial puppy food (88.45 %) and 2.5 % IEMS level (87.44%) as compared to 7.5 % (84.85 %) and 10 % (84.05 %) level of inclusion of IEMS however, it was comparable with 5 % level of IEMS inclusion. The *in vitro*ether extract digestibility was significantly (P<0.05) highest in 2.5 % level of IEMS (97.07%) and significantly (P<0.05) lowest in commercial puppy feed (91.96 %). The *in vitro* crude protein digestibility was significantly(P<0.05) higherin the commercial puppy food, 2.5 %, 5 % and 7.5 % level of IEMS as compared to 10 % level of IEMS. The results of *in vitro* digestibility values of present study was in agreement with the previous studies conducted by different researcher on dog food (Biagi et al., 2016; Kaur, 2021, Kaur et al., 2021; Biswas, 2022)

CONCLUSION

Based on the result obtained from the present study, it can be concluded that infertile egg with shell is a good source of alternative protein and can be incorporated in the in the diet of Labrador puppy food at 2.5 and 5 % level based on the *in vitro* digestibility of nutrients.

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