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Effect of supplementing azolla and empty pea pod on growth performance, blood biochemical metabolites and meat quality in White Pekin ducks

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ABSTRACT

Strategic dietary inclusion of low cost plant derived protein source in duck feed is critical for sustainability of the duck farm. Empty pea pod and azolla are two promising protein sources used as animal feed. The aim of this study was to evaluate the effect of supplementing dried and grounded azolla (AP) and empty pea pods (PP) on growth, blood biochemical metabolites and meat quality in white pekin ducks. A total of 90 white pekin ducklings were randomly assigned to 3 treatment groups (Control, T_1 and T_2) comprising of three replicated pens. Inside each replicate pen, a total of 10 ducklings were housed under standard management practices. AP and PP was supplemented as top up feed @ 5% of basal diet to experimental birds under T_1 and T_2 groups, respectively from day 0 to 56. At the end of the trial (56 day), 3 birds from each pen were sampled for serum and breast muscle. Results reveled that AP and PP has significant influence on body growth. The effect on growth was in the order of: T_2>T_1>Control. It was also observed that PP had hypoglycemic and hypolipidemic effect on ducks. However, AP and PP supplementation did not affect carcass traits, *p*H, cooking loss and Lipid Peroxidase Activity (TBA value) in duck meat.Thus, it could be concluded that supplementation of dried pea pods (PP) might be of great benefit to white pekin ducks as a source of cheap and unconventional protein.

Keywords: Body growth, Dried Azolla, Meat quality, Pea pod, White pekin duck

Prospect of duck meat is increasing worldwide owing to the increased customer attraction for its higher fat content, distinct aroma, colour and taste (Ali *et al.* 2007). Changing food habit, expansion of frozen and value-added meat sector are also responsible for the popularity of the duck meat. In India, genetically improved white pekin ducks (*Anas platyrhynchos*) are gaining popularity among farming community due to assured marketing returns (Ghosh *et al*. 2021). Small farmers raise their ducks using rice-based diet with supplementation of vegetable residues (Naik *et al*. 2020) for cost optimization. However dietary protein remains the most crucial component in the diet for food animals (Liu *et al.* 2015). As the protein rich ingredients are costly and small farmers can hardly afford those for preparation of diets (Mavromichalis 2014), the composite feed prepared using rice by-products are obviously lower in crude protein content than the recommended level.

In this background, two inexpensive yet protein rich feed ingredients like azolla and empty pea pods might be a game changer to solve this problem. These two cheap alternative protein sources (Wadhwa *et al.* 2017) can be used in pekin duck feed to augment growth without extra cost involvement. Azolla and empty pea pod are rich in protein with balanced amino acid profile, minerals and bioactive compounds (Ghosh *et al.* 2018, Ravi *et al*. 2018). Azolla can be grown in ponds or in water tanks in backyard. Dried azolla may be available in all the seasons unlike fresh one and always better in terms of storage and preservation. On the other hand, empty pea pod is the by-product of agro-industries and generally considered as waste. But it has tremendous potential as livestock feed (Wadhwa *et al.* 2017). To the best of our knowledge, no scientific literature is available till date on usefulness of this incredible feed ingredient in the diet for white pekin ducks. Considering the above facts, present study was designed to investigate the effect of supplementing dried and grounded azolla and empty pea pods on growth, feed efficiency, biochemical metabolites and meat quality in white pekin ducks.

MATERIALS AND METHODS

Birds and housing: The experiment was conducted during February-April of 2022 at the experimental shed of Sasya Shaymala Krishi Vigyan Kendra (Farm Science Center), Ramakrishna Mission Vivekananda Educational and Research Institute, Kolkata. Day-old white pekin ducklings (90) of 'Vigova Super-M' strain were procured from Central Poultry Development Organization and Training Institute, Bengaluru, India. The ducklings were randomly assigned to three treatment groups, where each

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group comprised of 3 replicated pens. Inside each replicate pen, a total of 10 ducklings were housed. The individual pens (1.22 m × 0.76 m) were separated with wire nets and floors were covered with saw dust and chopped straw as litter material. Brooding was done for first two weeks using electric heating elements. Each pen was fitted with automated drinker and feeder. Birds were appropriately cared under guidelines laid down by the Institutional Animal Ethics Committee.

Feed preparation: A single diet approach was followed during the whole trial period (1-56 day). The basal diet (NRC 1994) was rice by-product based and resembled mash feed used by local duck growers in field. The composition and calculated nutrient content of the diet are presented in Table 1. The diet was made available to the birds *ad lib*.

Table 1. Formulation and calculated content of basal diet

Ingredient of feed	(%)
Broken rice	38.2%
Broken wheat	17.6%
Rice polish	8.5%
Soyabean	15%
DORB	10%
Fish meal	6%
DCP	2%
Calcite	1%
Sodium bi carbonate	0.2%
Salt	0.2%
Oil	0.2%
Vitamin premix	0.1%
Calculated content	
Crude protein	16.5%
Ether extract	4%
Crude fibre	4.3%
Calcium	3.5%
Available phosphorus	0.5%
ME (KCal/kg)	2500

Preparation of azolla and empty pea pod powder and subsequent analysis for proximate composition, minerals and bioactive compounds: Fresh azolla (*Azolla pinnata*) and empty pea pod (*Pisum sativum* var. *Bonneville*) were collected from instructional farm of the institute. Both the material were rinsed under tap water followed by de-mineralized water. Afterwards, these were shed dried for 5 days with an average moisture content of 15.7% and subsequently grounded using an electrical grinder (500 Watt). Azolla (AP) and Pea Pod (PP) powder were then sieved through 60 mesh size and stored in zip lock plastic bags under refrigeration. The powder materials were analyzed for proximate composition (Supplementary Table 1) as per AOAC (2000).

Natural pigments such as carotenoid and anthocyanin were analyzed as per methods described by Sadasivam and Manickam (1992) using spectrophotometer (Eppendorf BioSpectrometer®, kinetic). Minerals were estimated as per Allen *et al*. (1989). Briefly, the samples were digested with tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) and subsequently filtrate was analyzed using Atomic Absorption Spectrophotometer (Analyst 900 F, Perkin Elmer, Germany) for micro-elements (Fe, Cu, Zn, Mn). Carotenoids, anthocyanins and trace mineral contents are presented in Supplementary Table 1.

Treatments: The experiment was conducted with a control group (C) and two treatment groups (T_1 and T_2) with three replications. The birds in the control group were fed with only basal diet, while birds in the T_1 and the T_2 groups were fed with basal diet along with top up supplementation of AP and PP @ 5% of basal diet.

Growth performance: Body weight of the ducks was recorded individually using an electronic balance at weekly interval. Mortality was recorded as it happened.

Analysis of serum metabolites: At the end of trial (i.e. on 56th day), three birds from each pen were selected randomly (total nine birds per treatment) for slaughter. Blood samples (3 mL) were collected from each bird at slaughter. Serum was harvested by centrifugation of the blood at 1800 × g for 10 min at 4°C. Serum biochemical metabolites (glucose, total protein, aspartate aminotransferase, alanine transaminase, triglyceride, uric acid and creatinine) were analyzed using reagent kits (Erba, Germany) in a semi-automatic analyzer (ErbaChem 5X, Germany).

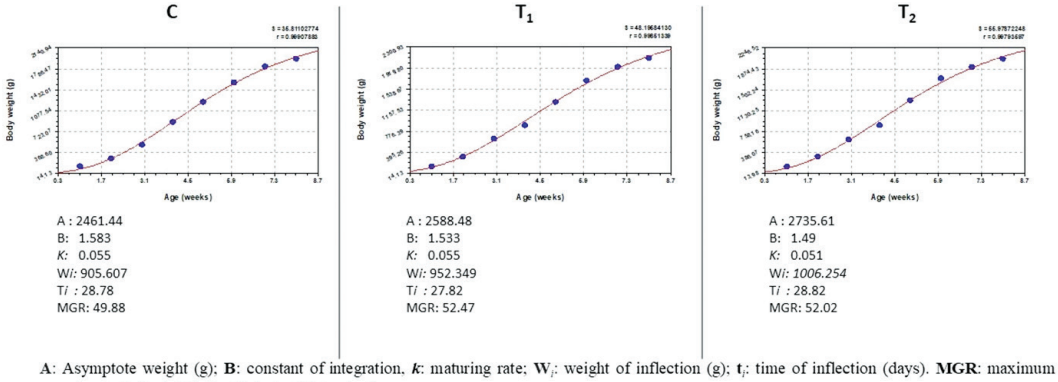
Carcass characteristics and meat quality: Birds were only allowed to access drinking water 12 h prior slaughter. Feed was completely withdrawn. Weight of individual birds was recorded after presenting at slaughterhouse and subsequently been slaughtered by severing the jugular vein using standard procedure. Carcass was defeathered by a motor driven de-feathering machine and then eviscerated manually. Dressing percentage was calculated by the following formula:

$$\text{Dressing \%} = [(\text{Dressed weight} \div \text{Live weight}) \times 100]$$

Weight of the cut-up parts (Breast, thigh, drumstick, wing, neck, giblets) from the carcass was duly recorded by digital weighing balance.

Physical meat quality (*p*H, cooking loss) was determined from breast (pectoralis major) meat. The *p*H values of meat samples (about 15 g) were measured on day 1 and day 7 using a handheld digital *p*H meter (EUTECH, UK). Cooking loss of the meat samples was analyzed according to Abdulla *et al.* (2017). Lipid peroxidation of the meat samples was measured according to Witte *et al.* (1970) on day 1 and day 7 and it was expressed as MDA (mg)/kg meat. All the meat samples were analyzed in triplicates.

Statistical analysis: Data were statistically analyzed by one-way ANOVA using SPSS program (SPSS 20.0, SPSS Inc., Chicago, USA). Means were compared by Duncan Multiple range Test (Duncan 1955). Body weight prediction curve was constructed by Curve Expert 1.4 program (Hyams 2010) for Windows using Gompertz's non-linear regression model.



A: Asymptote weight (g); **B**: constant of integration, ***k***: maturing rate; $\mathbf{W}_i$: weight of inflection (g); $\mathbf{t}_i$: time of inflection (days). **MGR**: maximum growth rate (g/day); $\mathbf{R^2}$: Coefficient of determination

Fig. 1. Growth parameters and growth curves of white pekin ducks under control (c) and treatment groups (T_1 and T_2).

The mathematical expression in Gompertz's model is:

$$W_t = Ae^{-eB-kt}$$

$W_i = A/e$; $t_i = B/k$; MGR = $W_i \times k$

Where, W_t , predicted weight (g); A, asymptote weight (g) when time goes to infinity; B, constant of integration; *k*, maturing rate (g/day); *t*, time unit (day), and *e*, natural constant (2.72). W_i ,weight of inflection; t_i, time of inflection and MGR, maximum growth rate (g/day).

RESULTS AND DISCUSSION

Growth performance: Highly significant (P=0.006) difference in body growth was observed across the treatments and control group in final body weight in white pekin ducks (Table 2). Highest body weight (2104.66 g) was achieved in groups (T_2) supplemented with PP followed by groups supplemented by AP (T_1) and control (C) group. Significantly (P<0.05) superior body growth in T_2 group was also noticed in second week, third week and sixth week. Acharya *et al*. (2015) showed that supplementation of fresh azolla has positive effect on growth of white pekin broiler ducks. Similarly, Ghosh *et al.* (2022) obtained better body weight (2003 g) in pekin ducks on 56 d when supplemented with fresh azolla @ 7 g per bird per day in homemade diet under backyard farming. In line with the previous works, present study clearly demonstrated that top up supplementation with empty pea pod (PP) and dried azolla (AP) significantly (P=0.006) improved final body weight of pekin ducks on 56 d under confinement.

The growth parameters for control and treatment groups, obtained from Gompertz's growth curve, are shown in Fig. 1. Asymptote weight (A) value calculated in the present study is 2461.44 g, 2588.48 g and 2735.61 g respectively for Control, T_1 and T_2 groups. It is the maximum weight that could be achieved by white pekin ducks when the time tends to infinity. Inflection points deduced from the Gompertz's equations are the spots in the growth curve to mark the shift from growth acceleration to retardation. In the present study, the highest infection weight (Wi) was recorded as 1006.25 g in T_2 group followed by T_1 (952.34 g) and Control (905.60 g). Effect of AP and PP supplementation was observed in maximum growth rate (MGR) of ducks. Highest MGR was observed in T_1 group (52.47 g/day) followed by T_2(52.02 g /day) and control (49.88 g /day)group. Protein is an important nutrient for production of food animals including birds. Present study revealed that both AP and PP contains good crude protein content (Table 2). PP is considered as widely available cheap vegetable industry waste in India and this byproduct contains essential amino acids, calcium and phenolic antioxidants (Kumari and Deka 2021). Substantial amount of trace minerals (Fe, Cu, Zn and Mn) had also been quantified in AP and PP under the present trail (Table 2). Trace minerals play critical roles in growth in

Table 2. Effect of AP and PP on body growth

Body weight (g)	Period	Treatment			SEM	P-value
		C	T_1	T_2		
	7 days	141.34	139.78	141.25	0.421	0.251
	14 days	272.19^b	317.88^a	318.9^a	5.916	0.000
	21 days	506.71^b	619.83^a	649^a	16.305	0.000
	28 days	891.5	878	892.66	15.06	0.916
	35 days	1234.06	1318.33	1313.66	21.588	0.208
	42 days	1561.33^b	1712.83^a	1701^a	28.842	0.047
	49 days	1830.5^b	1908.33^{ab}	1952.5^a	20.433	0.036
	56 days(Final)	1959.16^b	2055^a	2104.66^a	20.848	0.006

Means bearing different superscripts in a row differ significantly (P<0.05).

Table 3. Effect of AP and PP on serum metabolites

Parameter	Treatment			SEM	P-value
	C	T_1	T_2		
Glucose	161.29	154.10	145.36	3.889	0.259
Total Protein	4.69	4.81	4.43	0.154	0.619
Aspartate transaminase (SGOT)	60.33	52.83	51.5	2.352	0.269
Alanine transaminase (SGPT)	32.83	34.33	31.83	1.146	0.695
Triglyceride	11	11.83	9.66	0.493	0.201
Uric Acid	6.86	7.08	8.28	0.485	0.466
Creatinine	0.3	0.26	0.24	0.01	0.275

Means bearing different superscripts in a row differ significantly (P<0.05).

Table 4. Effect of AP and PP on carcass characteristics

Parameter	Treatment			SEM	P Value
	C	T_1	T_2		
Dressing Percentage (%)	67.73	65.36	65.67	0.834	0.478
Breast (g)	598.72	664.32	723.1	14.986	0.097
Thigh (g)	261	275.83	272.66	4.174	0.325
Drumstick (g)	135	146	145.66	2.328	0.082
Wing (g)	201.66	197.68	204.83	1.962	0.348
Neck (g)	121.83	126	124.5	2.128	0.745
Liver (g)	39.83	42.83	38.72	1.183	0.379
Heart (g)	12.66	11.91	13.41	0.335	0.195
Gizzard (g)	62.33^{ab}	59^{c}	65^{a}	0.885	0.011

Means bearing different superscripts in a row differ significantly (P<0.05).

meat type ducks (Wang *et al.* 2020). Considering all these facts, present study suggests that PP in particular could be utilized as growth additive in white pekin duck feed as a promising waste derived protein.

Serum metabolites: Serum metabolites (glucose, total protein, aspartate amino transferase, alanine transaminase, triglyceride, uric acid and creatinine) were analyzed within 24 h after harvesting serum from blood. Although the present study found non-significant (P<0.05) results (Table 3) in serum metabolites across the experimental groups, it was observed that PP has slight hypoglycemic and hypolipidaemic action on pekin ducks. This might be attributed to the bioactive compounds present in PP (Table 2). Coman *et al.* (2020) described bioactive roles of different fruit and vegetable peels in animal models. A plethora of fruit and vegetable wastes demonstrated hypoglycemic and hypolipidemic effect owing to the presence of anthocyanin, phenolic acids and flavonoids (Oliveira *et al.* 2020). However, in the present study, all the serum metabolites were in normal range indicating no harmful effect of either AP or PP in the diet.

Carcass characteristics and meat quality: Carcass characteristics are presented in Table 4. The relative dressing percentage was in the order of Control>T_2>T_1. However, there was no significant (P>0.05) difference in dressing percentage and cut up parts except gizzard across the groups. Irrespective of the statistical differences, our study revealed higher dressing percentages than the previous works. Kokoszyński *et al.* (2019) reported 66.5% dressing percentage in P33 Pekin duck strain. Starčević *et al.* (2021) found 63.9% and 63.6% dressing percentage in Star 53 White Pekin duck strain under intensive and semi-intensive system respectively. The higher dressing yield of experimental ducks (Vigova Super M strain) in the present study might be due to strain difference. The highest weight of the breast meat was observed in T_2 group followed by T_1 and Control group (P=0.097). Further, it was revealed that the PP supplementation increased the gizzard weight (P = 0.011) in T_2 group.This might be attributed due to the presence of insoluble dietary fibre content in PP.

Physio-chemical quality of meat (pH_{24hrs}, cooking loss, $TBA_{day1,}$ and TBA_{day7}) is presented in Supplementary Table 2. AP and PP supplementation did not affect (P = 0.748) pH, cooking loss and Lipid Peroxidase Activity (TBA value) in duck meat.The mean *p*H values of meat samples were 5.58, 5.57 and 5.6 in control, T_1 and T_2 groups respectively. pH_{24hrs} determined in the present study were lower than the observations found by Starčević *et al.* (2021). This might be due to different pre-slaughter condition and different glycogen content in the muscle at slaughter. Cooking loss is an important indicator for predicting meat characteristic during processing and value addition (Pang *et al.* 2020). The highest cooking loss was noticed in T_2 group (35.61%) followed by control (35.5%) and T_1 (34.16%). Cooking loss (%) observed in this experiment was in agreement with the results obtained by Ghosh *et al.*(2022) in white pekin ducks grown in backyard farming condition.TBA value [expressed as MDA (mg)/kg] is the indicator of lipid peroxidase activity in meat (Fernández *et al.* 1997) and widely used for estimating self-life of processed meat and meat products.In the present experiment, non-significant (P>0.05) difference in TBA values was observed among three treatment groups in both day 1 and day 7 post slaughter. This indicates dietary supplementation of both AP and PP has no influence on shelf-life of breast meat in white pekin ducks.

In conclusion, this study clearly demonstrated that both dried azolla and pea pod powder could enhance growth performance of white pekin ducks. Supplementation of empty pea pod powder (5% as top up feed) produced maximum body weight in this experiment. Pea pod powder also tends to decrease glucose and triglyceride in blood of experimental birds. However, its supplementation did not influence carcass characteristics and meat quality. Large quanta of empty pea pod can be easily obtained from hotels or processing centers. Further processing of pea pod powder from empty pea pod is also a simple and cost-effective method. Overall, pea pod powder could be used as a low cost yet potent organic additive in feed of white pekin duck @5% top up for optimum growth performance.

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