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Original article

Prospects of *Poikelocercus pictus* (Orthoptera: Acrididae) as an alternative protein source for Rhode Island Red chicken

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ABSTRACT

Objectives: Insects have great potential as an alternative nutrient source in animal feed, fisheries, and poultry industries for sustainable development. *Poikelocercus pictus* (PP) is an edible grasshopper that has a high percentage of crude protein; thus, we evaluated it as an alternative protein source for Rhode Island Red (RIR) chicken.
Methods: We fed RIR chicken on a commercial diet (control) and PP diets: T_1 (PP meal throughout the trial, T_2 (PP diet for the last 30 days of the trial), T_3 (PP diet for the last 15 days of the trial). We maintained eight RIR chickens in four groups each with six replications.
Results: Feed analysis indicated significantly higher crude protein levels ($P < 0.05$) in the PP diet than in the commercial diet. The feeding trial showed that PP diet feeding for 15 days did not record a significant difference with control for feed intake, food conversion ratio (FCR), and growth. RIR chicken demonstrated significantly higher feed intake and gained higher weight when fed on the PP diet as compared to the control ($P < 0.05$). Maximum FCR was observed when chickens were fed the PP diet for 45 days (3.21 ± 0.03).
Conclusion: Our study indicated that the grasshopper species, *Poikelocercus pictus*, might serve as a protein source in poultry feed. Our results suggest that PP diets could replace or be used as a partial substitute for conventional plant-based protein sources in poultry feed.

1. Introduction

Human population growth coupled with the climate change scenarios has made the shortage of protein resources for animal feed a major challenge for the future of the poultry industry worldwide (Lamsal et al., 2019; Elahi et al., 2022; Pan et al., 2022). Insects as protein alternatives (food and feed ingredients) have been proposed over conventional feed due to high feed conversion efficiency and being eco-friendly (Jonas-Levi and Martinez, 2017; Poma et al., 2017). Grasshoppers, crickets, and locusts could serve as a potential protein source for poultry due to higher digestibility and good quality high protein content (DeFoliart et al., 1982; Ramos-Elorduy, 1997; Wang et al., 2007).

Grasshoppers have shown high (687.7 g/kg) crude protein and crude fat (73.5 g/kg), crude fibers (66.2 g/kg), and ash content (40.8 g/kg) (Das and Mandal, 2014). Grasshoppers have 52–77 % proteins (Ramos-Elorduy et al., 1984). Grasshoppers have about 64% protein and thus could be used in the poultry industry as part of the feed (Rodríguez-Rodríguez et al., 2022).

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Earlier studies have shown that Rhode Island Red (RIR) chickens demonstrated better growth when fed on insects (Khan, 2018). FCR of indigenous chicken improved when the fish meal was replaced with the grasshopper meal (Nginya et al., 2019). The literature review asserts the significance of grasshoppers as a potential source of protein in commercial feed for poultry. Thus, we evaluated the *Poekilocerus pictus* diet (PP diet) for Rhode Island Red Chickens.

2. Materials and methods

2.1. Birds and their management

Rhode Island Red (RIR) chickens were obtained from the hatchery of the Poultry Research Institute (PRI), Rawalpindi and were maintained at Government Poultry Farm, Sook Kalan, Gujrat, Punjab, Pakistan. Chickens were put in pens randomly with a density of eight birds per pen ($\approx$3ft^2/chick). The pens were having a perch assembly (50 cm from the floor) made up of wood (Khawaja et al., 2012a,b; 2013a,b).

Chicks were maintained under recommended conditions of temperature (34 ± 01 °C) during 1st week and then, we lowered the temperature to 3 °C/week. The pens were provided with at least nine hours of light per day (maximum 14 h). Feed and water provision was *ad libitum* consumption and chicken vaccination program was followed as per guidelines for the breed and area (Khawaja et al., 2013a,b).

2.2. Collection of Grasshoppers

Adult grasshoppers were collected from pesticide-free fields selected by personal interview survey of the community of Deva Vatala National Park (DVNP), Bhimber. Only those sites were selected which have a history of no pesticide usage for at least the last five years. Specimens were identified by using standard taxonomic keys for the Acridids (Bughio et al., 2014; Gupta and Chandra, 2016).

Grasshoppers were deprived of food for 24 h before killing and processing. Wings and legs were removed and the bodies were dried under a hot air oven at 40 °C. Then, the dried grasshoppers were milled for feed formulation.

Table 1
Composition (g/kg) and proximate composition of the formulated diets (%).

Ingredients	Commercial diet (control)	PP diet
Corn	570.00	540.00
Soybean Meal	305.10	–
Meat Bone Meal	71.00	71.00
Soybean Oil	32.10	32.05
Limestone	0.59	0.59
Celite	10.00	10.00
Salt	2.62	2.62
Sodium Bicarbonate	0.88	0.88
L-Lysine	2.60	2.60
DL-Methionine	2.93	2.93
L-Threonine	0.54	0.54
Premix	1.50	1.50
Ronozyme ProAct	0.20	0.20
P. pictus	–	305.10
Proximate composition (% of dry matter)		
	Commercial Diet	**PP Diet**
Crude protein	55.20 ± 0.75	65.92 ± 0.75
Crude lipid	09.75 ± 0.52	02.54 ± 0.16
Carbohydrate	04.24 ± 0.15	09.30 ± 0.15
Ash	15.39 ± 0.52	04.42 ± 0.18
Crude fibre	15.42 ± 1.18	17.82 ± 0.35

2.3. Composition of feed

The standard commercial feed ingredients were obtained from Ahsan Feed Mills, Tiwana Wala Pea, Gujranwala, Punjab and feed were prepared (Table 1) by following the National Research Council's recommendations (Council, 1994).

2.4. Experimental design

The experiment was laid out in a Completely Randomized Design (CRD) with six replications. A day-old 192 unsexed chickens were then randomly divided into four groups based on feed treatment: T_0 (control group: fed on a commercial diet throughout the trial), T_1 (PP diet throughout the trial (45 days), T_2 (PP diet for last 30 days of trial), and T_3 (PP diet for last 15 days).

2.5. Estimation of feeds ingredients

For proximate analysis of experimental feeds, 50 g/Kg of diet from each treatment was collected and replicated thrice. Crude lipid (using Soxhlet apparatus), crude protein (using Kjeldahl method), and ash content (using the muffle furnace at 550 ± 50 °C for about 6 h) were determined (Horwitz, 2010; Kim et al., 2017). Before protein estimation by spectrophotometry, the samples were kept at room temperature for at least half an hour. The absorbance of samples was measured at 595 nm. Carbohydrate content was calculated by difference method (100 − sum of protein, fat, and ash). Nitrogen-free extract (NFE) was calculated by subtracting the sum of crude protein, fat, ash and fibre from 100. Moisture content was determined by heat drying of the samples at 105 °C for 4 h.

2.6. Growth performance

Growth performance was measured by feed intake and weight gain. We weighed chickens before the start of experiment and then on the 15th, 30th, and 45th days. Weight of chicks was recorded after 12 h fasting and feed conversion ratio (FCR) was determined by formula:

$$\text{FCR} = \text{Total feed intake}/\text{total weight gain}$$

2.7. Hematological and biochemical analysis

In the morning before feeding, we collected blood samples from the wing veins of chicks at the end of the 15th, 30th, and 45th day. Six samples of blood were taken from each group. cholesterol, protein, calcium, glucose, triglyceride, alkaline phosphatase, and uric acid were analyzed. A 5 ml blood sample in centrifuge tubes having heparin was centrifuged at 3000 rpm for 15 min. Serum samples were stored at −20 °C till further chemical analysis. Complete blood count (Hb, WBCs, RBCs, MCV, HCT, MCH, MCHC, and Platelets) was analyzed with an automated Hematology Analyzer (model HKTE0112 Guangzhou Hekang, China).

Commercial kits and diagnostic examinations were used to measure the biochemical characteristics of blood calorimetrically on a UV–visible spectrophotometer. Total protein was detected and quantified using the colorimetric method. Glucose and total cholesterol concentration were quantitatively measured with the help of the enzymatic colorimetric method.

2.8. Meat analysis

Chicks were slaughtered on 45th day to collect meat from breasts and thighs which was then dried, and ground for analysis i.e., crude protein, dry matter, moisture percentage, fat content, and total ash.

Table 2
Feed intake, weight gain, and FCR of RIR Chicken after the 15th, 30th, and 45th days.

Parameters	Duration (days)	T_0 (Commercial diet- Control)	T_1 (PP diet for 6 weeks)	T_2 (PP diet for 4 weeks)	T_3 (PP diet for 2 weeks)
Weight gain (g)	15	174.67 ± 4.16^{bc}	195.25 ± 3.72^{a}	181.66 ± 4.55^{b}	179.22 ± 5.65^{b}
	30	193.33 ± 3.76^{d}	216.85 ± 4.21^{a}	212.22 ± 4.12^{b}	204.67 ± 3.61^{c}
	45	209.00 ± 5.18^{d}	301.00 ± 4.84^{a}	246.22 ± 5.16^{b}	223.67 ± 4.25^{bc}
Feed intake (g/chick)	15	453.00 ± 4.78^{c}	547.18 ± 7.28^{a}	482.33 ± 4.38^{b}	472.00 ± 3.78^{bc}
	30	567.33 ± 3.48^{c}	696.64 ± 4.56^{a}	668.00 ± 3.85^{b}	621.12 ± 4.28^{c}
	45	627.67 ± 6.52^{d}	793.33 ± 5.45^{a}	763.42 ± 4.26^{b}	717.52 ± 7.34^{c}
FCR	15	2.59 ± 0.03^{c}	2.80 ± 0.01^{a}	2.66 ± 0.09^{b}	2.63 ± 0.08^{bc}
	30	2.93 ± 0.04^{c}	3.17 ± 0.03^{a}	3.15 ± 0.02^{a}	3.03 ± 0.01^{b}
	45	3.00 ± 0.01^{cd}	3.21 ± 0.03^{a}	3.14 ± 0.05^{ab}	3.10 ± 0.06^{c}

Means with different superscripts $^{(a, b, c, d)}$ in a row differ significantly ($P < 0.05$).

2.9. Statistical analysis

Analysis of variance (ANOVA) was performed to determine the significance level and means were compared by applying Tukey's post hoc test where effects were significant ($P < 0.05$) *Statistix* Software.

3. Results

3.1. Feed analysis

The estimation of feed compositions showed crude protein content of PP (65.92 ± 0.75%) was higher as compared to commercial diet (55.20 ± 0.75%). However, crude lipid content (09.75 ± 0.52) and ash (15.39 ± 0.52) were higher in commercial diet (Table 1).

3.2. Growth performance of RIR chicken

In this study, we assessed the growth performance of RIR chickens by feeding them PP diet diets for two, four and six weeks in comparison with commercial feed (control) using feed intake, body weight gain, and FCR (Table 2).

3.3. Feed intake (g)

Chicken showed significantly greater feed intake when fed on PP diet as compared to commercial feed ($P < 0.05$). RIR chicken fed on PP diet for six weeks consumed significantly more feed ($P < 0.05$) as compared to commercial diet (Table 2). After 15 days, maximum feed intake was recorded in PP diet (547.18 ± 7.28) as compared to other treatments. Similarly, maximum feed intake was observed PP diet after 30 days (696.64 ± 4.56) and 45 days (793.33 ± 5.45) (Table 2).

3.4. Weight gain (g)

The average weight gain by Rhode Island Red chicks after two, four and six weeks showed significant variations ($P < 0.05$). We recorded that, after 15 days, RIR chicken fed with PP diet gained an average body weight of 195.25 ± 3.72 g. Similarly, after 30 days, significantly higher weight gain was recorded in chicken fed on PP diet (T_1) 216.85 ± 4.21 g as compared with control (193.33 ± 3.76). The lowest body weight gain was observed in control (Table 2).

3.5. Feed conversion ratio (FCR)

RIR chicken showed significant differences in FCR between all treatment groups ($P < 0.05$). The highest FCR was recorded in chicken fed on PP diet (T_1) after 15 days (2.80 ± 0.01), 30 days (3. 17 ± 0.03) and 45 days (3.21 ± 0.03). We observed that FCR was higher in chickens after six weeks as compared to four weeks and two weeks (Table 2).

3.6. Meat analysis

Data on meat analysis of RIR Chicks showed significant differences ($P < 0.05$) in dry matter, crude protein, and crude fat (Table 3). In breasts, we observed that RIR chickens showed better perfor-

Table 3
Comparative meat composition of RIR Chicken.

Parameters	Breast					Thighs				
	T_0	T_1	T_2	T_3	P value	T_0	T_1	T_2	T_3	P value
Dry Matter	26.93 ± 0.75^{b}	28.39 ± 0.35^{a}	28.17 ± 0.78^{a}	27.94 ± 0.67^{a}	0.0002	27.07 ± 1.63	27.21 ± 1.26	26.28 ± 1.15	26.37 ± 1.39	0.1343
Crude Protein	78.57 ± 1.14^{c}	83.02 ± 0.63^{a}	81.17 ± 1.62^{b}	79.90 ± 1.58b^{c}	0.0001	71.58 ± 1.24^{a}	69.20 ± 2.32^{b}	68.19 ± 2.44^{bc}	67.60 ± 2.3	0.0001
Crude Fat	6.24 ± 0.24^{c}	7.30 ± 0.18^{a}	6.76 ± 0.22^{b}	6.61 ± 0.16^{bc}	0.0001	17.50 ± 0.99^{a}	16.56 ± 0.51^{b}	16.15 ± 0.27^{bc}	15.61 ± 0.69^{c}	0.0001
Total Ash	4.54 ± 0.86	4. 71 ± 0.13	4.77 ± 1.14	4.65 ± 0.91	0.4314	4.53 ± 1.02	4.67 ± 0.95	4.56 ± 0.94	4.44 ± 0.98	0.2931

Means with different superscripts $^{(a, b, c, d)}$ in a row differ significantly ($P < 0.05$).

Table 4
Hematological parameters (Mean ± SE) of Rhode Island Red Chicks.

Hematological Parameters	T_0	T_1	T_2	T_3	P value
HCT (%)	32.19 ± 0.02	31.90 ± 0.07	32.26 ± 0.06	31.33 ± 0.03	0.0735
Haemoglobin (g/dL)	9.36 ± 0.03	9.30 ± 0.01	09.42 ± 0.04	09.37 ± 0.02	0.1047
MCH (%)	30.56 ± 0.08	30.76 ± 0.10	33.50 ± 0.06	30.46 ± 0.04	0.0981
MCHC %	28.40 ± 0.07	28.23 ± 0.12	28.76 ± 0.16	28.12 ± 0.05	0.0009
MCV (fL)	107.97 ± 0.26	111.23 ± 0.31	109.60 ± 0.23	110.40 ± 0.48	0.0001
Platelets (×103/μL)	6.33 ± 0.03	4.33 ± 0.01	4.66 ± 0.03	1.66 ± 0.01	0.0023
RBC (×106/μL)	3.10 ± 0.01	3.15 ± 0.02	2.77 ± 0.03	3.05 ± 0.02	0.0019
WBC (×103/μL)	242.33 ± 0.78	239.67 ± 1.31	243.12 ± 0.94	240.32 ± 1.42	0.0032

Table 5
Biochemical parameters (Mean ± SE) of Rhode Island Red Chicks showed non-significant differences ($P > 0.05$).

Biochemical parameters	T_0	T_1	T_2	T_3	P value
Cholesterol (mg/dL)	131.33 ± 1.71	126.53 ± 1.05	128.42 ± 1.32	127.25 ± 1.06	0.5853
Glucose (mg/dL)	220.71 ± 2.54	224.66 ± 1.20	219.33 ± 1.78	222.17 ± 1.36	0.1206
Triglyceride (mg/dL)	530.48 ± 1.09	533.83 ± 1.30	528.62 ± 0.85	525.67 ± 0.76	0.2310
Alkaline Phosphate (U/L)	1022.13 ± 4.09	1025.42 ± 3.21	1017.14 ± 1.62	1018.20 ± 2.16	0.0863
Protein (g/dL)	4.50 ± 0.04	4.59 ± 0.04	4.61 ± 0.03	4.47 ± 0.07	0.0652
Uric Acid (mg/dL)	4.15 ± 0.01	4.06 ± 0.01	4.04 ± 0.02	4.12 ± 0.04	0.0693
Calcium (mg/dL)	10.12 ± 0.58	10.23 ± 0.25	10.19 ± 0.53	10.21 ± 0.26	0.1475

mance when fed on PP diet as compared to control. Crude fat also showed significant variations in means between treatments. Maximum crude fat was recovered when chicks were fed PP diet for six weeks (T_1). Similarly, crude protein was significantly different among treatments.

In thigh meat, we observed significant differences in crude protein and cure fat content ($P < 0.05$). Significantly higher means were recorded in T_1 as compared to other PP diets and control. Ash content showed non-significant differences in means in both type of meat i.e., breast and thighs.

3.7. Hematological analysis

Data showed non-significant variations ($P > 0.05$) in hematological parameters i.e., HCT (%), Haemoglobin (g/dL), MCH (%), MCHC %, Platelets ($\times 10^3/\mu L$), RBC ($\times 10^6/\mu L$), and WBC ($\times 103/\mu L$) among four treatments (Table 4).

3.8. Biochemical analysis

Results demonstrated that Cholesterol (mg/dL), Glucose (mg/dL), Triglyceride (mg/dL), Alkaline Phosphate (U/L), Protein (g/dL), Uric Acid (mg/dL), and Calcium (mg/dL) indicated non-significant differences ($P > 0.05$) (Table 5). Calcium and cholesterol levels in the blood serum of RIR chicks showed non-significant differences ($P > 0.05$).

4. Discussion

We explored potential of *Poekilocerus pictus* as an alternative protein source to raise RIR chicken (Khawaja et al., 2012a,b). Locusts, grasshoppers, and crickets have been explored for their nutritional qualities, rearing possibilities for commercial purposes and biomass production (Van Huis 2020). We observed a higher value of crude protein in the PP diet as compared to the control. Feed intake and mean weight gain were significantly higher for the PP diet as compared to the commercial diet.

RIR chicken showed better FCR when fed on the PP diet (T_1) as chicken grew in age as compared to other treatments and FCR increases as the age of chicken progress (Haque et al., 1999) and FCR becomes better due to greater physical activities (Khawaja et al., 2012a,b).

A similar trend in feed intake was reported in an earlier study where sequential feeding with diets varying in protein and energy contents indicated a higher preference for insect-based diets and greater FCR was recorded as compared to commercial diets (Nascimento Filho et al., 2020). The higher protein and fat content of insect-based diets make them more palatable to birds (Rodríguez-Rodríguez et al., 2022).

Similar trends of growth performance of RIR chickens on the insect-based protein were recorded (Khawaja et al., 2012a,b). Insects contain highly valuable compounds with a positive effect on animal's immune systems that promote their growth (Gasco et al., 2018).

Dry matter, crude fat, and crude protein exhibited significant differences among treatments in breast meat but total ash showed non-significant differences. Thighs muscles have high dry matter content than breast muscles and total ash content not varying significantly in both breast and thighs (Fujimura et al., 1996). Biochemical parameters showed non-significant variations ($P > 0.05$) in mean among all treatments (Akbarian et al., 2011; Khawaja et al., 2013a, b; Kokore et al., 2021). We detected no differences in serum cholesterol levels of RIR chicken among four treatments. Similar results were communicated in two different studies (Bhatti et al., 2002; Khawaja et al., 2012a,b and 2013a,b) who explained that prelaying and post laying conditions did not affect the biosynthesis of cholesterol. RIR chicken like other breeds possess similar regulatory mechanisms for calcium, cholesterol, triglyceride, glucose, protein, and concentrations (Khawaja et al., 2013a,b).

We observed non-significant differences in the hematological parameters of RIR Chicken among different treatments. Recent studies reported that hematological parameters may vary with breed genotype, feed type, feeding habits, feed supplements, and conditions but not necessarily differ significantly (Alam et al., 2020; Duah et al., 2020; Muneer et al., 2021). This study emphasizes that RIR chicken has shown significantly better growth on the PP diet than on a commercial diet.

5. Conclusion

RIR chicken showed better food intake and better body weight gain when fed on the PP diet as compared to the control diet with improved FCR. The meat composition in breasts and thighs showed significant differences between crude protein and crude fat among treatments. There is an indication that the insect-based diet (PP diet) could be a potential protein source in chicken diets. This study asserts that insect-based diets may be explored extensively as an alternative to conventional diets for sustainable poultry production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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