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Optimization of Dietary Energy and Protein Levels for Reproductive Performance of Native Chicken Variety (Aseel cross)

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ABSTRACT: Native chicken production is becoming popular in recent years. Energy and protein requirements of Aseel varieties and its crosses could be different from those of commercial layers. Recent rise in cost of feed ingredients included in poultry diets leads to find cost effective measures in feeding. The aim of the study was to optimize dietary energy and protein requirements for reproductive performance in native chicken variety (Aseel cross). Nine experimental diets were formulated with three different energy levels (2400,2300,2200 kcal ME) each with three protein levels (14,13,12 %) and fed to the birds from 21-60 weeks of age. Fertility, Hatchability and Embryonic mortality were calculated and analysed from 29-60 weeks. The effect of interaction due to dietary energy and protein had no significance (P>0.05) on fertility, hatchability on total eggs and hatchability on fertile eggs from 29 to 52 weeks of age. After 52 weeks, low energy treatment group (2200 ME Kcal/Kg and 12%) had significantly (P<0.05) lower fertility and hatchability than other treatment groups. The embryonic mortality during incubation had no effect due to interaction of energy and protein levels upto 48 weeks of age. Though there was no significant effect, it was concluded that optimum level of energy and protein for reproductive performance is 2400 ME Kcal/Kg and 12 % CP diet.

Keywords: Native chicken variety, Energy, Protein, Fertility, hatchability and Embryonic Mortality.

INTRODUCTION

Aseel is one of the well-known indigenous chicken breeds of India and is known for its known for its pugnacity, majestic gait, high stamina, and dogged fighting qualities (Singh, 2001). Indigenous chicken production is becoming popular due to higher demand for their meat and eggs because of its flavour, low fat content and non-usage of antimicrobial growth promoters. The purchasing power and growing population increases demand of native chickens particularly higher in urban areas and cities.

At present condition in India, egg production grew at the rate of 6.16% annually from 2008-09 to 2018-19 and 8.51% in 2019 with a total of 103.32 billion eggs produced in the year 2018-19. The per capita availability of eggs in the country was 79 eggs per annum in 2019 (BAHS 2019). Native chickens contributed about 14.4% to the total egg production of the country in the year 2016.

The College of Poultry Production and Management at Hosur (CPPM), is developing a chicken variety from the local Aseel chicken population and its crosses. With the rising cost of nutrients, an appropriate energy and protein level is most important factor to reduce feed cost per unit of weight gain (NRC, 1994). Non-availability of specific feed and higher feed cost are the major constraints faced by the farmers rearing native chickens intensively and an optimized energy level maybe chosen while formulating diet for male and female Aseel native chickens (Kumaravel *et al*., 2021).

Fertility and Embryonic mortality during incubation period is an indicator of deficiency or inadequacy of the breeder diets (Leeson *et al*., 1979). Aminafshar *et al*. (2015) that energy level and type can be effective on changing blood lipid metabolites such as cholesterol and triglyceride. Selection of appropriate levels of protein and energy is required without affecting the fertility and hatchability of native chickens. Hence, the present study was carried out to optimize dietary energy and protein requirements for reproductive performance in the newly developing native chicken variety at CPPM, Hosur.

MATERIALS AND METHODS

Experimental birds. A total of 486 females of native chicken variety were received from CPPM farm complex at 21 weeks of age and divided in to nine dietary treatments. Each treatment was given with six replicates and in total 54 replicates were assigned to present study. In each replicate two male birds were distributed in the male female ratio of 1:5 of the same age group. The experimental design was 3 × 3 factorial design. The study period started during January, 2021 and ended on September, 2021. Birds were reared under deep litter system of management following standard management practices. The birds in each treatment experimental feed and ad libitum water was provided. This study was carried out at College of Poultry Production and Management at Hosur (CPPM), Tamil Nadu Veterinary and Animal Sciences University, India.

Experiment diets (21-60 weeks of age). During the study period from 21 to 60 weeks of age, the nine dietary treatments with six replicates of nine birds in each. In each replicate two male birds were distributed in the male female ratio 1:5. Nine experimental diets were formulated with three different energy levels (2400, 2300, 2200 kcal ME) each with three protein levels (14, 13, 12 %) and fed to the female birds throughout the experimental period from 21-60 weeks of age. Feed consumption was recorded on weekly basis. The ingredient and calculated nutrient composition of diets for breeder phases are given in the Table 1.

Table 1: Ingredient and Nutrient Composition of experimental diets (%) with different energy and protein concentration's (21-60 weeks).

Ingredients	2400 Kcal ME/Kg			2300 Kcal ME/Kg			2200 Kcal ME/Kg		
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Maize	42.60	43.20	44.43	34.90	36.05	37.32	27.85	28.95	30.02
Soybean meal	10.65	7.30	4.34	8.85	5.83	2.87	7.40	4.35	1.30
DORB	37.00	40.00	41.50	46.80	48.50	50.00	55.15	57.00	58.90
Salt	0.35	0.31	0.24	0.16	0.23	0.32	0.32	0.31	0.32
Shell grit & Calcite	7.20	7.10	7.10	7.10	7.10	7.10	7.11	7.15	7.14
Dicalcium phosphate	0.90	0.91	0.94	0.88	0.91	0.94	0.85	0.85	0.86
Lysine powder	0.31	0.18	0.44	0.32	0.38	0.44	0.33	0.39	0.45
Methionine powder	0.10	0.11	0.12	0.10	0.11	0.12	0.10	0.11	0.12
Vitamin and other supplements*	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Total	**100.0**	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutrient Composition (%)									
ME K.cal/kg	2401	2401	2400	2300	2300	2300	2200	2200	2200
Crude Protein	14.01	13.00	12.00	14.00	13.00	12.00	14.00	13.00	12.00
Lysine	0.85	0.65	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Methionine	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Calcium	3.00	2.96	2.96	2.96	2.96	2.96	2.96	2.96	2.96
Available Phosphorous	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Crude Fibre	7.84	8.13	8.23	9.07	9.19	9.29	10.10	10.25	10.40

*Vitamins and other supplements included at 0.66 per cent which includes UltraviteM @ 0.25%, AB2D3K @ 0.02%, Pytase@ 0.006%, Choline chloride @ 0.07%, US curatox @ 0.10%, NSP enzyme @ 0.05%, Meriplex powder @ 0.014%, liver tonic@ 0.01% & sodium bicarbonate @0.1%.

Collection of Data. Eggs were collected twice daily from the experimental groups and the collected eggs were sorted, fumigated and stored for seven days throughout the study period of 29 to 60 weeks of age. Appropriately labelled hatching eggs were set in setter and transferred to hatcher on 18th day on weekly basis from each treatment group. Percent fertility and hatchability were calculated using the formula:

$$\text{Per cent fertility} = \frac{\text{Number of fertile eggs}}{\text{Total number of eggs set}} \times 100$$

Per cent hatchability on total eggs

$$= \frac{\text{Number of chicks hatched}}{\text{Total number of eggs set}} \times 100$$

Per cent hatchability on fertile eggs

$$= \frac{\text{Number of chicks hatched}}{\text{Total fertile eggs set}} \times 100$$

All unhatched eggs were inspected for embryonic development and the embryo mortality were described as Early (EEM), Mid (MEM), Late (LEM) embryonic mortality and unhatched chicks after day 21. All the values were calculated in percentage.

Statistical analysis. Recorded data was subjected to Statistical analysis using SPSS (2018) software for two-way ANOVA and level of significance was measured using Tukey's test following the procedure of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Fertility. Fertility rate among the different dietary energy and protein groups is presented in the Table 2. Results revealed that there were no significant effects due to interaction of varying levels of energy and protein on fertility during the trial period of 29-60 weeks. The lesser values of fertility in T7, T8, T9 during 53-56 weeks and T8 and T9 during 57-60 weeks were either due to decreased or zero egg production during that phase. The birds might have entered in to moulting due to feeding of low energy diets (2200 ME Kcal/Kg) or depletion of body reserves. Similar trend was observed by Pearson and Herron (1981) that the consistent low rate of egg production by lowest energy allowance of 1.52 MJ AME/day (363KCal) was due to inadequate allowance to meet energy requirements of the bird's egg production. Significant (P<0.05) effect due to different levels of energy was found in high energy treatment than medium and low energy treatment groups after 52 weeks of age. The same trend was observed in different protein level treatment groups.

Table 2: Effect of different levels of Energy and Protein on Fertility.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	89.22±0.26	89.41±1.28	89.65±1.58	90.56±2.75	70.30±1.12	87.49±1.83	85.66±1.17	91.12±0.67
T2	88.85±1.89	92.85±1.19	92.99±1.68	88.89±2.84	87.53±2.95	91.03±1.36	84.61±1.60	89.03±0.79
T3	90.79±1.59	92.27±0.85	90.52±1.82	91.67±2.04	83.52±2.63	93.62±0.85	90.47±0.72	89.71±0.41
T4	91.20±1.26	90.05±0.69	88.35±1.33	91.11±2.08	77.19±1.51	91.97±2.45	95.69±1.62	90.21±0.87
T5	91.14±2.54	86.12±3.82	90.00±1.38	90.56±2.92	69.02±2.49	90.64±1.89	88.72±0.38	88.34±0.70
T6	91.19±2.49	90.12±0.82	93.18±1.44	92.22±1.97	82.29±2.12	90.16±1.07	91.06±1.05	88.10±0.89
T7	93.40±0.72	87.76±2.67	90.33±1.13	89.30±2.88	79.28±2.36	90.86±1.12	58.52±1.84	86.19±1.00
T8	90.93±2.36	91.28±0.66	88.47±1.23	86.85±2.56	86.29±2.13	90.39±1.10	59.31±1.05	54.87±0.69
T9	86.49±2.86	90.87±1.02	90.03±0.68	87.87±1.24	87.59±2.67	79.17±1.79	28.05±0.67	20.45±0.69
Effect of Energy levels								
2400 ME Kcal	89.62±1.14	91.29±1.34	91.06±1.27	90.37±1.81	80.45±4.17	90.71±1.61	86.92±3.82	89.95±5.3
2300 ME Kcal	91.18±1.14	89.03±1.34	90.51±1.27	91.30±1.81	76.17±4.17	90.93±1.61	91.82±3.82	88.89±5.3
2200 ME Kcal	90.27±1.14	89.97±1.34	89.61±1.27	88.01±1.81	84.39±4.17	86.81±1.61	48.68±3.82	47.02±5.3
Effect of Protein levels								
14 %CP	91.28±1.14	89.22±1.34	89.45±1.27	90.32±1.81	75.59±4.17	90.11±1.61	79.95±3.82	89.17±5.3
13 %CP	90.31±1.14	89.81±1.34	90.48±1.27	87.76±1.81	80.95±4.17	90.69±1.61	77.55±3.82	77.14±5.3
12 %CP	89.49±1.14	91.27±1.34	91.24±1.27	90.59±1.81	84.47±4.17	87.65±1.61	69.91±3.82	59.27±5.3

* Means in columns not sharing a common superscript differ significantly (P<0.05)

Hatchability on total egg set basis. The results of hatchability on total egg set basis due to different dietary treatments are presented in Table 3. The interaction of protein and energy levels did not have any influence over the hatchability on total egg set basis throughout the trial period from 29 weeks to 52 weeks. But during 53 to 56 weeks and 57 to 60 weeks, T4 (86.1 % and 77.47%) showed significant (P<0.05) effect on hatchability (TES) than T9 (47.12% and 15.91%). Though other treatment groups showed difference in hatchability, they were not significant whereas other treatment groups except T8 (47.05%) were significantly different from T9 (15.91%) group during 57-60 week period.

These results are in contrast with the findings of Saleh *et al*. (2017) who found that poor and best hatchability was observed on high energy with low protein (2800 ME Kcal/Kg and 14% CP) diet and high energy with high protein (2800 ME Kcal/Kg and 18% CP) diet respectively in FUNAAB alpha hens (Improved variety). The reason could be due to low productivity of our variety chickens, the energy and protein was sufficient to produce fertile eggs and good hatchability. Significant (P<0.05) hatchability difference noticed during 53-56 and 57-60 week periods were due to birds had a pause or almost ceased to produce after 52 weeks of age and more of infertile eggs in the low energy and low protein treatment group (2200 ME Kcal/Kg and 12% CP) were observed.

No effects on fertility and hatchability of total eggs set was found for the different energy treatment groups from 29-56 weeks but 57-60 week period shown significantly lower hatchability (44.47%) in low energy (2200 ME Kcal/Kg) than medium (2300 ME Kcal/Kg) and high energy (2400 ME Kcal/Kg) treatment groups. The results are in agreement with the Van Emous *et al*. (2015), who reported that the breeders fed high metabolizable energy diet showed an increased hatchability of fertile eggs compared to birds fed low metabolizable energy diet during second phase of lay (45-60 weeks). Similar trend was observed in different protein levels that only during 57-60 week period high protein diet (14%) had significant effect on hatchability (75.23% vs 55.04 %) compared to low CP (12%) diet.

Hatchability on fertile egg set basis. The results of hatchability on fertile egg set are presented in Table 4. There was no effect due to interaction of dietary energy and protein levels during the entire period on hatchability of fertile eggs set except during 57-60 week period where T9 had significantly lower hatchability (19.44%) than other treatment groups T1 to T7 (87.57 % to 81.94 %). A poor hatchability was noticed in low energy and low protein diet groups after 56 weeks of age. This result was due to very less number of egg production and more of infertile eggs in those treatment group (2200 ME Kcal/Kg and 12 % CP) after 52 weeks of age. Conversely, Sun and Coon (2005) reported that egg production was not affected in breeders fed a high energy and low energy diet (2,970 vs 2,816 ME Kcal/Kg) during the entire laying period. The poor results in the present study might be due to the energy levels which was very low when compared to their high and low energy levels.

Table 3: Effect of different levels of Energy and Protein on Hatchability of Total eggs set.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	81.38±2.54	75.77±2.96	74.98±6.86	80.83±5.79	71.00±3.44	75.59±4.42	72.08±1.99^{ab}	77.78±1.10^{b}
T2	77.88±2.38	82.40±2.26	83.94±6.65	79.58±6.81	80.32±1.53	78.51±2.82	69.06±2.83^{ab}	75.35±0.74^{b}
T3	83.32±2.26	84.42±1.79	78.28±5.09	82.50±4.83	82.67±1.43	83.26±2.11	79.49±1.03^{ab}	76.33±0.88^{b}
T4	82.80±1.49	78.18±0.69	74.84±3.11	80.42±4.48	79.90±0.82	82.35±5.97	86.19±0.92^{b}	77.47±1.94^{b}
T5	81.81±3.43	74.58±6.72	79.77±2.67	76.67±5.57	74.02±2.2	81.68±3.09	76.56±1.16^{ab}	71.56±0.28^{b}
T6	84.34±4.75	78.20±1.72	84.85±2.39	81.25±3.99	82.51±0.57	79.40±2.51	78.76±1.62^{ab}	72.88±1.15^{b}
T7	84.04±2.11	72.85±7.48	80.51±6.38	76.67±6.19	80.12±0.64	80.67±1.50	75.51±1.23^{ab}	70.44±0.94^{b}
T8	80.36±3.40	81.36±2.13	76.29±3.13	72.86±5.18	73.97±0.55	83.69±1.98	72.80±1.86^{ab}	47.05±15.75^{ab}
T9	74.37±1.68	78.90±2.16	79.26±1.44	73.09±2.86	73.61±1.12	74.11±3.52	47.12±1.65^{a}	15.91±15.91^{a}
Effect of Energy levels								
2400 ME Kcal	80.86±1.64	80.87±2.20	79.07±2.67	80.97±3.01	78.00±2.12	79.24±1.95	73.54±4.26	76.49±4.35^{b}
2300 ME Kcal	82.98±1.64	76.99±2.20	79.82±2.67	79.44±3.01	78.81±2.12	81.14±1.95	80.47±4.26	73.97±4.35^{b}
2200 ME Kcal	79.59±1.64	77.70±2.20	78.69±2.67	74.21±3.01	75.90±2.12	79.49±1.95	65.14±4.26	44.47±4.35^{a}
Effect of Protein levels								
14 %CP	82.74±1.64	75.60±2.20	76.78±2.67	79.31±3.01	77.01±2.12	79.66±1.95	77.92±4.26	75.23±4.35^{b}
13 %CP	80.02±1.64	79.45±2.20	80.00±2.67	76.37±3.01	76.10±2.12	81.29±1.95	72.81±4.26	64.66±4.35^{ab}
12 %CP	80.68±1.64	80.51±2.20	80.80±2.67	78.95±3.01	79.60±2.12	78.92±1.95	68.43±4.26	55.04±4.35^{a}

* Means in columns not sharing a common superscript differ significantly ($P<0.05$)

Table 4: Effect of different levels of Energy and Protein on Hatchability of fertile eggs set.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	89.39±2.09	84.66±2.09	82.46±6.35	89.06±3.73	81.30±2.54	85.35±3.32	80.41±1.14	85.57±0.70^{b}
T2	86.78±1.32	88.71±1.49	88.71±3.75	87.56±4.88	88.62±1.03	86.58±1.81	79.35±1.22	84.12±0.09^{b}
T3	90.68±1.92	91.47±1.05	85.97±2.01	89.79±3.29	89.18±1.09	89.47±1.55	87.55±0.41	84.99±0.63^{b}
T4	89.89±1.40	86.57±0.43	84.62±0.91	87.93±2.90	87.92±0.59	89.51±4.51	90.62±0.25	86.36±1.35^{b}
T5	88.37±2.24	84.53±4.38	88.15±0.57	85.27±3.50	83.69±1.59	90.24±2.46	86.55±0.40	80.93±0.66^{b}
T6	91.53±3.17	86.39±1.13	90.59±0.49	88.08±2.59	89.84±0.31	87.05±1.87	86.89±0.25	82.99±0.76^{b}
T7	91.20±1.23	82.14±6.33	87.98±1.04	84.99±4.45	88.04±0.36	88.57±0.25	84.34±2.52	81.94±0.99^{b}
T8	87.81±2.01	89.21±1.69	85.37±0.52	81.80±3.51	83.48±0.68	91.81±2.92	84.66±1.99	56.93±19.04^{ab}
T9	83.41±1.86	87.36±1.41	88.44±0.24	81.98±2.14	84.35±0.79	92.26±4.49	62.76±1.30	19.44±19.44^{a}
Effect of Energy levels								
2400 ME Kcal	88.95±1.15	88.28±1.65	85.71±2.14	88.80±2.04	86.36±1.58	87.14±1.67	82.44±4.93	84.89±5.25^{b}
2300 ME Kcal	89.93±1.15	85.23±1.65	87.79±2.14	87.09±2.04	87.15±1.58	88.94±1.67	88.02±4.93	83.43±5.25^{b}
2200 ME Kcal	87.47±1.15	86.24±1.65	87.26±2.14	82.92±2.04	85.29±1.58	90.88±1.67	77.25±4.93	52.77±5.25^{a}
Effect of Protein levels								
14 %CP	90.16±1.15	84.46±1.65	85.02±2.14	87.33±2.04	85.76±1.58	87.81±1.67	85.12±4.93	84.62±5.25^{b}
13 %CP	87.66±1.15	87.48±1.65	87.41±2.14	84.88±2.04	85.26±1.58	89.54±1.67	83.52±4.93	73.99±5.25^{ab}
12 %CP	88.54±1.15	88.40±1.65	88.34±2.14	88.61±2.04	87.79±1.58	89.60±1.67	79.07±4.93	62.47±5.25^{a}

* Means in columns not sharing a common superscript differ significantly ($P<0.05$)

There was a difference of 60% hatchability between T9 and other treatment groups both in total egg set (TES) and fertile egg set (FES) basis after 56 weeks of age. These data indicate that the major effect of low dietary energy and CP on hatchability is in fact related to production and fertility. This is observed by the lack of difference in embryonic mortality in various stages of incubation. High energy diet with low CP diet resulted in improved hatchability on TES and FES in the experimental period upto 56 weeks of age. Similar to this findings, Lopez and Leeson (1995) reported an increased fertility and hatchability of total eggs set and decreased embryonic mortality during the entire laying period when birds received a low CP.

No significant effect was noticed on hatchability of FES due to different energy levels from 29 weeks to 56 weeks whereas 57-60 week period showed significantly lower hatchability (52.77%) in the low energy treatment (2200 ME Kcal/Kg) group than medium (2300 ME Kcal/Kg) and high energy (2400 ME Kcal/Kg) groups (83.43% and 84.89%). Anne and Herron (1981) also reported that hatchability decreases at higher protein allowance combined with decreased energy allowance.

Similarly, different protein levels had no impact over the percentage of hatchability on FES. Sometimes the hatchability percentage in the birds fed low CP diets was little higher and comparable to the high protein diets up to 52 weeks of age. From 57-60 week period onwards high CP diets had significantly higher hatchability than low CP diets (12%). Though there was no significant difference in hatchability during 53-56 weeks and hatchability started decreasing from 53 weeks of age in low energy and low protein treatment groups. The results of Lopez and Leeson (1995) that the birds fed lower dietary protein showed consistent improvement in hatchability of eggs throughout the 40 wk trial period and the major advantage of low protein diets is improving fertility without affecting hatchability

Embryonic Mortality

Early embryonic mortality. The results for embryonic mortality and unhatched chicks for the trial (29-60 weeks) are presented in Tables 5-8. From 29 to 48 weeks of age there was no significant effect due to interaction between energy and protein levels on embryonic mortality. During 49-52 weeks of age low energy and protein group (2200 ME Kcal/Kg and CP 12%) had significantly lower early embryonic mortality than other treatment groups T1 and T5. Again from 53-60 weeks of age, there was no significant effect due to interaction of energy and protein levels on embryonic mortality. In the similar manner, early embryonic mortality was significantly (P<0.05) higher (3.30%) in high (2400 ME Kcal/Kg) energy treatment diets than low energy (2200 ME Kcal/Kg) diets (2.27%) only during 49-52 weeks of age. Though this is in contrast with the reports of Saleh *et al*. (2017) that the early embryonic mortality was not affected by the high energy with low protein (2800 ME Kcal/Kg and 14% CP) diet and high energy with high protein (2800 ME Kcal/Kg and 18% CP) diet, the effect was noticed for a sheet period of four weeks. Different protein levels had no effect on early embryonic mortality for the whole experimental period from 29 weeks to 60 weeks.

Table 5: Effect of different levels of Energy and Protein on Early Embryonic Mortality from 29 weeks to 60 weeks of age.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	2.55±0.56	4.33±0.82	4.49±1.04	1.92±0.82	4.07±0.61	4.17±1.0.81^{b}	3.99±1.58	3.57±1.57
T2	3.72±0.32	2.54±0.46	3.27±1.16	3.32±1.01	1.72±0.22	3.04±1.34^{ab}	6.03±2.86	4.80±0.98
T3	2.95±0.44	2.80±0.44	3.77±0.53	2.76±0.57	4.08±0.88	2.68±0.33^{ab}	4.28±0.89	5.84±0.94
T4	2.04±0.29	3.68±0.51	4.86±0.95	2.77±0.56	2.50±0.18	2.75±0.69^{ab}	2.16±0.40	4.34±1.08
T5	2.91±0.53	4.66±1.48	3.08±0.49	2.87±0.96	3.83±0.43	3.48±0.75^{b}	3.94±1.08	5.97±2.08
T6	2.47±0.63	4.61±0.55	2.84±0.70	2.29±0.52	2.57±0.38	2.56±0.91^{ab}	2.89±0.30	4.54±1.08
T7	2.27±0.40	4.33±1.79	3.96±1.79	3.68±0.55	2.68±0.17	3.06±0.08^{ab}	3.87±0.95	4.06±1.36
T8	2.31±0.86	3.62±0.67	4.08±1.03	5.24±0.61	2.44±0.31	3.75±1.01^{ab}	5.19±1.51	4.02±1.18
T9	3.90±0.54	3.47±0.89	2.41±0.29	2.50±0.32	4.93±0.42	2.89±1.01^{a}	4.20±2.27	2.78±2.29
Effect of Energy levels								
2400 ME Kcal	3.07±0.31	3.22±0.56	3.84±0.57	2.67±0.40	3.29±0.72	3.30±0.59^{b}	4.77±0.94	4.74±1.32
2300 ME Kcal	2.47±0.31	4.14±0.56	3.60±0.57	2.65±0.40	2.97±0.72	2.93±0.59^{ab}	3.00±0.94	4.95±1.32
2200 ME Kcal	2.83±0.31	3.81±0.56	3.48±0.57	3.81±0.40	3.35±0.72	2.27±0.59^{a}	4.42±0.94	5.95±1.32
Effect of Protein levels								
14 %CP	3.07±0.31	3.22±0.56	3.84±0.57	2.67±0.40	3.29±0.72	3.30±0.59^{b}	4.77±0.94	4.74±1.32
13 %CP	2.47±0.31	4.14±0.56	3.60±0.57	2.65±0.40	2.97±0.72	2.93±0.59^{ab}	3.00±0.94	4.95±1.32
12 %CP	2.83±0.31	3.81±0.56	3.48±0.57	3.81±0.40	3.35±0.72	2.27±0.59^{a}	4.42±0.94	5.95±1.32

* Means in columns not sharing a common superscript differ significantly (P<0.05)

Mid embryonic mortality. Effect of interaction of different energy and protein levels on mid embryonic mortality was not significant upto 52 weeks of age and thereafter T7 and T1, T6 and T8 had significantly higher mid embryonic mortality during 49-52 weeks and 57-60 weeks respectively than other dietary treatment groups. It has also been reported by Daghir, and Shah (1973) that the requirements of vitamin B12 and pyridoxine were increased in high protein intakes. Wilson (1997) reported progression of mortality was seen in Vitamin B12 deficiency for all the three weeks of incubation but our results are not consistent. when different protein levels were considered high protein (14%) had significantly higher mid embryonic mortality only during 49-52 weeks period of the whole trial period from 29 to 60 weeks. Leeson and Summers (2009) observed, lower hatchability in breeders fed a low energy and high protein diet and the decrease appear to be resulted from increased embryonic mortality in mid incubation and unhatched pips. Similarly, high energy (2400 ME Kcal/Kg) had higher (P<0.05) mid embryonic mortality only during the last four weeks of the trial period (57-60 weeks). The embryonic mortality during this mid incubation is a sensitive indicator of nutritional deficiencies in the breeder diet (Leeson *et al*., 1979).

Late embryonic mortality. There was no effect of diets due to different energy and protein levels on late embryonic mortality from 29 to 60 weeks of age. The same trend was observed in different energy levels for the whole period of 29-60 weeks. The protein levels also did not any show effect on the late embryonic mortality. The results could be due to adequacy of vitamins and Minerals such as Vitamin D and magnesium in the breeder diet, otherwise the deficiency of those resulted in late embryonic mortality (Wilson, 1997)

Unhatched chicks. Among the interaction of different energy and protein levels 33-36 week, 49-52 week and 57-60 week periods showed significant (P<0.05) effect on unhatched chicks. In different energy levels high percentage of unhatched chicks in medium energy treatment group (2300 ME KCal/Kg) during 33-36 weeks and 49-52 weeks along with high energy treatment groups. The protein levels had no effect on the unhatched chicks. Though the results are inconsistent through the different periods, deficiency of folic acid and excess of iodine (Wilson, 1997) and depletion of glycogen reserves (Menge *et al.*, 1979) could have attributed to higher unhatched chicks.

Table 6: Effect of different levels of Energy and Protein on Mid Embryonic Mortality from 29 weeks to 60 weeks of age.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	2.32±0.67	2.66±0.67	1.49±0.63	1.92±0.82	2.88±0.59	2.03±0.70^{bc}	4.52±1.73	3.41±1.57^{b}
T2	2.51±0.94	2.73±0.20	0.90±0.89	1.91±0.82	1.68±3.78	0.00^{a}	2.87±1.17	2.21±0.19^{ab}
T3	1.47±0.22	1.72±0.26	1.73±0.35	0.94±0.54	1.27±0.27	1.72±0.29^{b}	1.84±0.65	2.75±0.09^{ab}
T4	1.83±0.74	2.51±0.41	1.66±0.59	1.40±0.47	2.45±0.13	1.95±1.14^{b}	1.11±0.67	1.66±0.58^{a}
T5	3.63±1.33	2.32±0.53	1.63±0.43	2.82±0.58	2.48±2.9	0.00^{a}	1.06±0.63	1.74±0.19^{a}
T6	1.70±0.69	1.83±0.22	1.67±0.35	2.29±0.52	1.42±0.23	3.03±0.31^{bc}	3.52±0.26	3.53±0.48^{b}
T7	2.55±1.44	2.98±1.34	0.82±0.81	1.89±1.22	2.43±0.23	5.31±0.70^{c}	3.83±1.72	2.99±0.29^{ab}
T8	2.10±1.32	1.72±0.16	2.21±0.13	1.89±0.69	4.71±0.41	0.00^{a}	1.09±1.09	1.44±1.10^{a}
T9	4.23±1.22	2.32±0.44	1.84±0.49	4.99±0.64	2.65±0.32	0.00^{a}	1.92±1.02	2.78±2.78^{ab}
Effect of Energy levels								
2400 ME Kcal	2.10±0.59	2.34±0.34	1.37±0.57	1.59±0.42	1.94±0.59	1.25±0.30	3.08±0.71	2.79±0.64^{b}
2300 ME Kcal	2.39±0.59	2.22±0.34	1.65±0.57	2.17±0.42	2.11±0.59	1.66±0.30	1.90±0.71	2.31±0.64^{ab}
2200 ME Kcal	2.96±0.59	2.34±0.34	1.62±0.57	2.92±0.42	3.26±0.59	1.77±0.30	2.28±0.71	1.92±0.64^{a}
Effect of Protein levels								
14 %CP	2.23±0.59	2.72±0.34	1.32±0.57	1.74±0.42	2.59±0.59	3.10±0.30^{b}	3.15±0.71	2.69±0.64
13 %CP	2.75±0.59	2.26±0.34	1.58±0.57	2.21±0.42	2.96±0.59	1.86±0.30^{a}	1.68±0.71	1.32±0.64
12 %CP	2.47±0.59	1.95±0.34	1.75±0.57	2.74±0.42	1.78±0.59	1.58±0.30^{a}	2.43±0.71	3.02±0.64

* Means in columns not sharing a common superscript differ significantly (P<0.05)

Table 7: Effect of different levels of Energy and Protein on Late Embryonic Mortality from 29 weeks to 60 weeks of age.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	2.50±0.85	3.64±0.67	3.23±0.41	2.80±0.59	5.09±1.19	2.82±0.69	3.39±0.57	3.85±0.55
T2	2.85±0.88	2.69±0.70	2.30±0.97	2.82±0.62	3.48±0.38	4.45±0.33	3.82±2.00	1.91±0.29
T3	2.06±0.53	2.55±0.26	1.85±0.70	1.87±0.78	1.04±0.25	1.72±0.29	2.83±0.07	2.75±0.99
T4	2.57±0.55	2.70±0.41	2.84±1.15	3.71±0.83	3.40±0.11	2.37±0.96	3.19±0.64	3.29±0.23
T5	3.49±0.62	3.28±1.09	2.60±0.26	3.30±0.53	3.36±0.46	1.96±1.15	5.44±2.75	5.22±0.56
T6	1.54±0.64	2.45±0.21	2.71±0.59	3.67±0.82	2.72±0.07	3.35±0.33	3.83±0.46	2.79±1.26
T7	2.10±0.54	4.76±1.59	2.36±1.43	1.53±0.54	2.10±0.06	3.06±0.08	5.92±1.94	4.92±2.2
T8	4.40±0.85	2.51±0.66	2.14±0.78	4.79±1.03	2.44±0.31	2.78±2.78	2.40±1.09	0.00±0.00
T9	3.90±0.54	1.47±0.28	2.17±0.81	4.03±0.82	4.79±0.36	7.74±4.49	2.27±2.27	4.41±0.90
Effect of Energy levels								
2400 ME Kcal	2.47±0.39	2.96±0.45	2.46±0.50	2.50±0.43	3.20±0.59	3.00±1.07	3.35±0.71	2.83±0.52
2300 ME Kcal	2.53±0.39	2.81±0.45	2.72±0.50	3.56±0.43	3.16±0.59	2.56±1.07	4.15±0.71	3.76±0.52
2200 ME Kcal	3.46±0.39	2.91±0.45	2.22±0.50	3.45±0.43	3.11±0.59	4.53±1.07	3.53±0.71	1.64±0.52
Effect of Protein levels								
14 %CP	2.39±0.39	3.70±0.45	2.81±0.50	2.68±0.43	3.53±0.59	2.75±1.07	4.17±0.71	4.02±0.52
13 %CP	3.58±0.39	2.82±0.45	2.35±0.50	3.64±0.43	3.09±0.59	3.06±1.07	3.89±0.71	2.37±0.52
12 %CP	2.50±0.39	2.16±0.45	2.24±0.50	3.19±0.43	2.85±0.59	4.27±1.07	2.98±0.71	1.85±0.52

* Means in columns not sharing a common superscript differ significantly (P<0.05)

Table 8: Effect of different levels of Energy and Protein on Unhatched chicks from 29 weeks to 60 weeks of age.

Treatments	29-32 WKS	33-36 WKS	37-40 WKS	41-44 WKS	45-48 WKS	49-52 WKS	53-56 WKS	57-60 WKS
T1	3.25±0.94	4.71±0.81[b]	8.32±4.87	4..30±1.69	6.65±1.22	5.64±1.37[b]	7.69±7.69	3.60±1.24
T2	4.14±0.77	3.33±0.51[ab]	4.82±2.81	4.39±2.58	4.51±1.64	5.93±0.44[b]	7.92±2.63	6.96±0.74
T3	2.84±1.19	1.47±0.49[a]	6.68±3.00	4.65±1.64	4.44±0.82	4.40±0.63[b]	3.49±0.54	3.66±0.133
T4	3.69±1.71	4.54±0.33[b]	6.02±2.04	4.19±1.25	3.72±1.62	3.42±1.89[a]	2.92±0.24	4.34±0.39
T5	1.60±1.20	5.22±1.35[b]	4.54±1.47	5.73±1.69	6.65±1.45	4.32±1.73[a]	3.00±1.78	6.14±1.52
T6	2.76±1.29	4.72±0.66[b]	2.19±1.14	3.67±0.82	3.46±0.27	4.00±0.87[b]	2.88±0.3	6.15±1.53
T7	1.88±0.69	5.79±1.70[b]	4.89±0.88	7.91±2.60	4.75±0.79	0.00±0.00[a]	0.96±0.96	6.09±2.04
T8	3.39±1.01	2.93±0.63[ab]	6.21±1.12	6.28±2.34	6.93±2.31	1.67±1.67[a]	4.03±2.77	7.04±4.39
T9	4.56±0.43	5.39±0.44[b]	5.13±1.45	6.51±2.06	3.27±1.67	0.00±0.00[a]	0.00±0.00	0.00±0.00
Effect of Energy levels								
2400 ME Kcal	3.41±0.63	3.17±0.51	6.61±1.34	4.44±1.12	5.20±0.82	5.32±0.68[b]	6.37±1.86	4.74±1.06
2300 ME Kcal	2.68±0.63	4.83±0.51	4.25±1.34	4.53±1.12	4.61±0.82	3.91±0.68[b]	2.93±1.86	5.54±1.06
2200 ME Kcal	3.28±0.63	4.70±0.51	5.4±1.34	6.90±1.12	4.98±0.82	0.56±0.68[a]	4.18±1.86	4.38±1.06
Effect of Protein levels								
14 %CP	2.94±0.63	5.01±0.51	6.41±1.34	5.46±1.12	5.04±0.82	3.02±0.68	4.22±1.86	4.68±1.06
13 %CP	3.05±0.63	3.83±0.51	5.19±1.34	5.47±1.12	6.03±0.82	3.97±0.68	5.86±1.86	6.71±1.06
12 %CP	3.88±0.63	3.86±0.51	4.67±1.34	4.94±1.12	3.72±0.82	2.80±0.68	3.41±1.86	3.27±1.06

* Means in columns not sharing a common superscript differ significantly (P<0.05)

CONCLUSION

Interaction effect of varying levels of dietary energy and protein had no significant effect on fertility, hatchability on total eggs and hatchability on fertile eggs from 29 to 52 weeks of age. Similarly, the main effects of different energy and protein levels were insignificant up to 56 weeks of age. Different dietary effects revealed significance after 52 weeks of age are due to either decrease or cessation of egg production in low energy (2200 ME Kcal/Kg) with different protein (14, 13, 12%) groups (T7, T8 and T9). The embryonic mortality in different stages of incubation due to different diets were inconsistent to make any conclusion. From the results it was concluded that optimum reproductive performance was achieved in high energy and low protein group (2400 ME Kcal/Kg and 12 % CP) during the layer phase of 21-60 weeks in native chicken variety. Further studies are warranted on various levels of energy and protein on extended period after 60 weeks of laying phase to reduce the feed cost without affecting reproductive performance in this native chicken variety.

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Conflict of Interest. None.

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