International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(2): 390-395

Received: 24-01-2017
Accepted: 25-02-2017

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Influence of dietary energy and protein levels on growth performance and some blood biochemical indices of growing indigenous (Ghungroo) pigs

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Abstract
The effect of dietary energy and protein levels on growth performance as well as changes in certain blood biochemicals were studied in growing indigenous (Ghungroo) pigs. Twenty-four piglets were divided into four groups *i.e.*, high protein-high energy (Gr-I), high protein-low energy (Gr-II), low protein-high energy (Gr-III) and low protein-low energy (Gr-IV). Four rations were prepared by maintaining 2 levels of protein and 2 levels of energy and fed to respective group. Body weight gain in both Gr-II and Gr-IV were significantly lower than other groups. Energy level had significant effect on daily body weight gain of the animals. All the blood constituents increase with advancement of age in all the groups. However, haemoglobin, glucose and total protein differs significantly between the groups and their values were found to be higher in Gr-I. These results indicated that the ration with low protein and high energy could be optimum to promote growth in growing indigenous piglets as well as to sustain the blood constituents within range.

Keywords: Blood biochemical indices, Energy level, Ghungroo, Growth performance, Protein level

1. Introduction
During the last few decades, swine production has continued to grow and flourish in both developed and developing countries in order to meet the marked increase in demand for pork with increased human population. In India, the popularity of animal protein is also stimulating the consumer interest for pork. The global demand for pig continues to rise and it remains the most widely consumed meat protein in the world as well as in India especially in north-eastern region. It is essential to rear and multiply high yielding early maturing animals as it can meet the protein requirements of common people at an affordable price. Therefore, pig plays an important role in India in general and in north-eastern region of India in particular where more than 90% of people are fond of pork as a source of protein. In north-eastern region of India, pigs are generally reared for meat production. Although crossbred pigs has higher growth rate and meat production capability, still most of the farmers prefer indigenous pigs due to their less feed consumption, resistance to diseases and more adaptability to environmental conditions. Recently Ghungroo, an indigenous breed of pig in north-east region have shown to be potential in respect of prolificacy and growth rate [1].

Economic and viable rearing of pigs depends upon the efficiency of feed utilization as feed accounts about 70-75% of total cost of swine production. Swine can convert fed more efficiently than any other livestock. It is therefore necessary to know about the principles of feeding of this important, meat producing animals. Thus, a carefully planned and efficient feeding programme is essential for successful swine production. To formulate an efficient balanced diet, the ratio of protein and energy must be properly maintained. Excess energy relative to protein content in the diet may result in higher fat deposition. Similarly, higher protein intake will supply energy to the animal by deamination since excess protein will be used as a source of energy. Further, excess protein may be harmful to the animal since it may cause enlargement of spleen, liver and kidney. Hence, a properly formulated feed must have a well-balanced energy to protein ratio.

The energy requirement for pigs reared under tropical condition might be lower than that recommended by National Research Centre (1998) [2, 3]. Pigs on a high protein diet gain weight faster and efficiently than pigs on a low protein diet [4, 5]. However, better growth performance was reported in pigs fed on low protein diets [3].

Average daily gain (ADG) and feed conversion ratio (FCR) in the pigs fed the diet with 20% crude protein were found to be better than those in the pigs fed the diet with 14% of crude protein in the diet. There was a linear relationship between dietary protein level and ADG [6, 7], Average daily gain (ADG), feed efficiency and daily calorie intake of pig was significantly improved by energy content of diet [8]. It was also reported that higher content of dietary energy tend to increase growth rate of the pig [9].

The study of biochemical profile of blood has received great significance as it serve as valuable guidelines in evaluating the nutritional adequacy of the diet and health status of the animal [10]. It was also reported that the dietary contents affect the blood profile of healthy animals [11, 12]. There are very meagre information on blood serum constituents of growing indigenous pigs fed different protein and energy diet. Hence, the present experiment was carried out to study the effect of dietary energy and protein levels on concentration of certain blood serum constituents in growing indigenous (Ghungroo) pigs.

2. Materials and methods

2.1 Experimental animals, housing and management

Twenty four indigenous (Ghungroo) pigs of about 10-12 weeks age with 12.47± 0.08 kg mean body weight irrespective of sex were used as experimental animal. Prior to the start of experiment the male animals were castrated by open method and all animals were conditioned for a period of 15 days during which they were fed standard diet, dewormed and vaccinated against the prevalent contagious diseases. The animals were housed in the experimental shed having sufficient provision of sunlight and fresh air. They were reared under standard hygienic and uniform manage mental conditions throughout the experimental period. Before starting the experiment, sheds were properly cleaned and disinfected with potassium permanganate solution. During the experimental periods, the pens were washed and cleaned every day before feeding and watering. Feeding and watering trough were cleaned regularly to avoid any digestive disturbances.

2.2 Experimental design

Table 1: Percentage composition and estimated nutrient density of the ingredients of different rations

Attributes	Parts			
	Ration-I	**Ration -II**	**Ration -III**	**Ration -IV**
Percentage composition of the ingredients				
Maize grain	72	45	72	42
Wheat bran	-	25	2	25
Rice polish	-	7	8	20
Soyabean meal	16	12	7	5
GNC	4	-	3	-
Fish meal	5	8	5	5
Mineral mixture	2	2	2	2
Salt	1	1	1	1
Total	100	100	100	100
Estimated Nutrient Density				
CP%	18.21	18.43	15.23	14.98
M.E. (Kcal/Kg)	3458	3258	3468	3268
C P ratio	1:190	1:177	1:228	1:218

After 15 days of conditioning period, all the animals were randomly divided into four different groups of six animals each having both male and female animals. Four different rations were prepared by maintaining two levels of protein (high protein-100% and low protein-75%) and two levels of energy (high energy-100% and low energy-75%) as per NRC (1998). The Percent compositions of ingredients in different rations are given in Table 1.

The compositions of mineral mixture are as follows: Calcium 28%, Phosphorus 5%, Sodium Chloride 23.5, Iron 0.35%, iodine=10 ppm, copper= 100 ppm, Manganese=200ppm, Cobalt= 50 ppm.

Experiment was in a 2×2 factorial design for a period of 90 days. Four different rations *viz.* rations-I, II, III and IV were fed to four respective group of animals *i.e.* high protein –high energy (Gr-I), high protein –low energy (Gr-II), low protein - high energy (Gr-III) and low protein – low energy (Gr-IV).

2.3 Data recording and sampling

The animals were weighed individually in the morning before feeding and watering to avoid any possible variation that might be due to intake of feed and water and expressed in Kilogram. The body weight was recorded on day 0 and at monthly interval for a period of 90 days using a platform balance after thoroughly securing the animals. The individual body weight of the pig was recorded to compute average daily gain (ADG) in body weight. The nutrient density in different experimental rations was calculated out from the estimated percentages of protein and energy along with metabolizable energy in different rations.

Blood samples were collected in morning hours before feeding and watering in sterilized vials containing heparin as anti-coagulant from each animal of different treatment group at monthly intervals for a period of 90 days *viz*. on day 0, 30, 60 and 90. The experimental animals were restrained in ventro-dorsal position and blood samples were collected directly from anterior venacava under aseptic condition by using 15-gauge 4 inches needle and transferred to 15 ml glass centrifuge tubes. The blood was allowed to clot at room temperature and was centrifuged at 3000 rpm for 15 minutes. The serum was separated, kept in plastic vials and stored in deep freeze at -20 ˚C for estimation of blood biochemical constituents. A fraction of blood was transferred to sterilize test tubes with anticoagulant EDTA for hemoglobin estimation and with anticoagulant sodium fluoride for blood glucose estimation. Blood glucose was estimated immediately after collection of sample.

2.4 Haemato-biochemical analysis

Haemoglobin was determined by standard Sahli's acid haematin method [13] and the result was expressed in g/dl of blood. Blood glucose was estimated by Folin-Wu method as described [14].

Serum samples were analysed by spectrophotometry for total protein, Albumin: Globulin ratio, blood urea, blood urea nitrogen, serum calcium, serum inorganic phosphorus with commercially available invitro diagnostic kits (Merck limited India, ShivSagar Estate A Mumbai-400018). The urea level was estimated by Diacetyl Monoxime (DAM) method [15]. The blood urea nitrogen was calculated out by multiplying the Blood urea content with the factor 0.467 and was expressed in mg/dl.

2.5 Statistical analysis

Statistical analysis of the experimental data was carried out by one-way a 2x2 factorial design [16].

3. Results and Discussion

3.1 Nutrient Density and Growth performance

The estimated percentage of protein and calculated metabolizable energy content in different experimental rations

are given in Table 2. As desired, the % of protein in high protein groups was higher by 3% than low protein groups. Similarly, the energy content in high-energy rations was higher than the low energy rations. The Calorie protein (CP) ratio ranged from 1:177 to 1:228 in different experimental groups.

Table 2: Effect of different level of protein and energy on growth performance of Ghungroo piglets during the experimental period.

Attributes	Groups			
	Group-I	Group-II	Group-III	Group-IV
Growth performance				
Initial BW (kg)	12.71 ± 0.92	12.48 ± 0.80	12.27 ± 0.08	12.42 ± 0.21
Final BW (kg)	36.25 ± 2.48	29.78 ± 0.76	34.90 ± 2.22	29.27 ± 1.96
Total BW gain(kg)	23.51 ± 1.84	17.31 ± 0.48	22.63 ± 1.92	16.85 ±1.35
ADG (g)	254.67^{a} ± 22.08	190.00^{b} ± 5.81	248.67^{ac} ± 3.10	185^{b} ± 16.45
Effect of protein and energy on average gain in body weight				
Source of variation	d.f	SS	MSS	F-value
Protein	1	181.500	181.500	0.091
Energy	1	24704.170	24704.170	12.428
Protein × Energy	1	1.500	1.500	0.001
Error	20	39756.670	1987.833	-

The average initial and final body weight, average total gain in body weight (kg) and rate of mean daily gain (g) in different experimental groups are presented in Table 2. No significant difference was observed in the initial body weight of different experimental groups but significant difference was observed in Average Daily Gain in body weight between the groups. No significant difference was recorded between Group-I and II. However, the level of energy had significant (P<0.05) effect on the average daily gain in body weight (Table 2).

The average daily gain in body weight in Gr-I was highest followed by Gr-III and no significant difference was observed between the groups. However, daily gain in body weight in Gr-II and Gr-IV were significantly lower than the other groups. The cost of concentrate mixture was considerably lower in Gr-III in comparison to other groups. Energy and protein intakes are important factors in determining the growth performance of pig and the level of energy had significant effect on daily gain in body weight of the animals. In the present experiment, the higher average daily gain in group III could be attributed to adequate energy in the diet since excess energy in the diet results in fat deposition. Similarly, the higher values in average daily gain in Group I could be due to adequate energy and protein in the diet. The findings of daily gain in body weight in the present experiment was in good agreement with other workers [8, 9, 17, 18, 19] who reported better gain in average daily gain in body weight of the animals with higher levels of energy in the diet. Many workers reported better growth performance in pigs on a low protein [20, 3, 21] reported better growth performance in indigenous Nizerian pigs on 12% protein and 3.1 M Cal/kg metabolizable energy than pigs on 16% protein.

3.2 Blood biochemicals

The effect of experimental treatments on blood biochemical is summarized in Table 3. The normal levels of all the biochemical parameters were observed within the normal range that correlates with the findings of Kaneko *et al.* [22]. The level of haemoglobin increased from 10.54 ± 0.48 to 14.84 ± 0.46 g/dl and the level of blood glucose has increased from 85.27 ± to 1.39 to 125.73 ± 0.96 mg/dl at 0 to 90 days in all the experimental groups of animals. The level of haemoglobin and blood glucose differs significantly (P<0.05) amongst the groups as well as between the days of the experiment. Significantly (P<0.05) higher level of haemoglobin (g/dl) and blood glucose were observed in Gr-I, II and III than the Gr-IV and the level of energy, protein and their interaction had a significant (P<0.05) effect on them. The mean values were highest in Gr-I and lowest in Gr-IV. However, the values of Hb were towards the increasing trend in different groups receiving low protein to high protein in the diet. Increased Hb level were observed with increased protein content in the diet [10]. The lower Hb value obtained in protein restricted gilts due to inadequate protein intake [23]. In the present study, the higher level of Hb could be due to increased protein in the diet. The concentration of Hb in the blood reflects the nutritional and health status of the animal.

Table 3: Effect of different level of protein and energy on various blood biochemical indices in growing Ghungroo pigs

Blood Constituents	Groups	Days			
		Day 0	Day 30	Day 60	Day 90
Haemoglobin(g/dl)	Gr-I	10.78±0.40	15.16± 0.37	16.03 ±0.76	16.06 ± 0.06*
	Gr-II	10.53±0.05	14.05± 0.54	14.10 ±0.29	14.53± 0.37
	Gr-III	10.47±0.46	12.96± 0.60	14.35±0.44	14.41 ±0.36
	Gr-IV	10.40± 0.59	12.29 ± 0.31	13.60 ± 0.47	14.40± 0.42*
Glucose(mg/dl)	Gr-I	84.97 ± 1.33	99.90 ± 5.15	123.07± 2.13	130.01 ± 0.64
	Gr-II	85.26± 4.39	93.12 ± 2.29*	120.74 ±10.30*	126.40 ± 0.41
	Gr-III	85.30 ± 1.44	99.00± 4.81**	121.47± 6.40	128.05 ± 0.42
	Gr-IV	85.56± 3.55	89.50± 0.59*	106.60 ± 0.94**	118.47±0.95**
Total Protein(g/dl)	Gr-I	8.02 ± 0.17	8.37± 0.21	8.44± 0.22	8.5± 0.21
	Gr-II	7.99 ± 0.14	8.1 ± 0.15*	8.16 ± 0.16*	8.2± 0.15*
	Gr-III	7.99 ± 0.14	8± 0.09*	8.05 ± 0.07*	8.07± 0.13*
	Gr-IV	7.97± 0.19	7.99 ± 0.10*	8.00± 0.17*	8.02± 0.17*
A: G ratio	Gr-I	5.02 : 3.00	5.44 :2.93	5.44 :3.00	5.92 :2.68
	Gr-II	5.02 :2.94	5.40 :2.70	5.42 :2.74	5.83 :2.37

	Gr-III	5.05 :2.94	5.30:2.70	5.36 :2.69	5.51 :2.56
	Gr-IV	5.07:2.89	5.23 : 2.76	5.27 :2.73	5.33 :2.69
Blood Urea(mg/dl)	Gr-I	18.00± 1.01	19.00 ± 0.58	19.68± 0.80	19.70± 0.51
	Gr-II	18.01± 0.72	18.85± 0.73	19.00 ± 0.92	19.20 ± 0.47
	Gr-III	18.03±0.58	18.50 ± 0.48	18.68± 0.59	18.85± 0.70
	Gr-IV	18.00± 0.61	18.455± 0.77	18.50 ± 0.81	18.58 ±0.52
BUN(mg/dl)	Gr-I	8.41± 0.47	8.87 ± 0.27	9.19± 0.37	9.20± 0.23
	Gr-II	8.41± 0.34	8.80± 0.33	8.87 ± 0.43	8.97 ± 0.22
	Gr-III	8.42±0.27	8.70 ± 0.22	8.72± 0.27	8.80± 0.32
	Gr-IV	8.41± 0.27	8.57± 0.36	8.64 ± 0.38	8.68 ±0.24
Calcium (mg/dl)	Gr-I	9.00 ± 0.44	9.22 ± 0.29	9.33± 0.24	9.50± 0.23
	Gr-II	9.01± 0.26	9.13± 0.26	9.16 ± 0.29	9.30 ± 0.33
	Gr-III	9.02 ±0.32	9.10 ± 0.32	9.13± 0.18	9.20± 0.35
	Gr-IV	9.00± 0.18	9.00± 0.18	9.09± 0.38	9.20 ±0.40
Phosphorus(mg/dl)	Gr-I	5.03 ± 0.27	5.03 ± 0.31	5.35± 0.31	5.50± 0.20
	Gr-II	5.02± 0.13	5.21± 0.18	5.21 ± 0.18	5.40 ± 0.23
	Gr-III	5.01 ±0.23	5.20 ± 0.25	5.20± 0.25	5.35± 0.18
	Gr-IV	5.00± 0.24	5.20± 0.11	5.20 ± 0.11	5.30 ±0.11

Values represent mean ± standard error (n=6)

*$P < 0.05$, ** $P < 0.01$ and *** $P < 0.01$ represent levels of significance

The higher level of blood glucose in high energy diet *i.e.* in the ration of Gr-I and Gr-III could be attributed to the rations having higher quantity of maize grain or cereal grain as a source of energy [24, 25, 26]. Since maize is very rich source in soluble carbohydrate, thus it provides higher energy to the animal. The higher level of blood glucose in Gr-II could be due to deamination of excess protein of the ration which ultimately provides energy to the animal.

The level of protein has slightly increased from day 0 to 90 in all the groups and no significant difference ($P<0.05$) was observed amongst the groups. Significantly ($P<0.05$) higher level of total serum protein was observed in higher protein (Gr-I and II) groups and the level of protein had significant ($P<0.05$) effect on it (Table 2), which was in agreement with the previous works [10, 27, 28]. The level of serum albumin (g/dl) has increased while serum globulin has decreased from day 0 to 90 in all the groups. No significant difference (P>0.05) was observed amongst the groups as well as between the days. The significantly ($P<0.05$) higher level of serum albumin (g/dl) and lower values of serum globulin were recorded in groups fed on higher protein content in the diet. These findings are in good agreement with the findings of previous researcher [29, 30]. The level of energy, protein and their interaction had no significant effect on level of serum globulin while only the level of energy had a significant ($P<0.05$) effect on level of serum albumin.

The mean level of blood urea (mg/dl) and blood urea nitrogen (mg/dl) ranged from 18.38±0.32 to 19.10±0.38 mg/dl and 8.57±0.15 to 8.91±0.18; and serum calcium (mg/dl) and phosphorus (mg/dl) (mg/dl) were ranged from 9.09±0.14 to 9.26±0.15 mg/dl and 5.19±0.09 to 5.30±0.09 mg/dl respectively in different experimental groups. No significant difference (P>0.05) was found amongst the groups as well as between the days and the level of energy, protein and their interaction had no significant effect on them.

Blood urea, blood urea nitrogen, serum calcium and serum inorganic phosphorus values are also highest in Gr-I and lowest in Gr-IV. Higher values of blood urea and BUN were observed in groups fed on rations having higher protein content, which could be due to increased protein catabolism. Since ureatelic animals like pigs excrete amino nitrogen derived from α- amino acid as urea after protein catabolism. Various studies have suggested that BUN is directly related to protein intake and protein quality [31, 32]. Kumar *et al.* (1980) also reported the great influence of dietary protein in blood urea concentration. The higher values of blood urea nitrogen (mg/dl) level observed in groups fed on higher protein diets, was in good agreement with previous findings [33, 34, 35, 27, 37]. Blood urea and BUN level decreased in individuals after ingestion of glucose as insulin reduces the amino acid concentration in blood. In the present study, the different dietary levels of energy had no significant effect on blood urea nitrogen level. This was in good agreement with the findings observed by earlier reporters [38, 39].

4. Conclusion

From the present study, it can be concluded that 75% protein and 100% energy as per NRC (1998) [40] was suitable for optimum growth performance of growing indigenous (Ghungroo) pigs without adversely affecting the blood biochemical constituents.

5. Acknowledgements

The authors are grateful to Director, National Research Centre (NRC) on Pig, Indian Council of Agricultural Research (ICAR), Rani, Guwahati for conducting the research work there. And to HOD, Department of Veterinary Biochemistry, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022 for doing the analysis of the biochemical constituents of blood. The authors also express their gratitude to the Director of Post Graduate Studies, AAU and the Dean, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati for providing necessary financial support to conduct the research work.

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