Effects of feeding varying levels of dietary crude protein and digestible energy levels on nitrogen retention, urinary and blood plasma constituents in pigs fed from 9 to 60kg.

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Abstract

This study was designed to investigate the effects and possible interactions of varying crude protein (CP) and digestible energy levels (DE) on Nitrogen Retention, (NR), Urinary and blood plasma constituents in pigs reared from 6 to 60 kg liveweight. One hundred and twenty eight Large White x Landrace pigs weighing 9±1.5kg were assigned to sixteen groups of eight pigs, comprising four females and four castrated males in a 4x4 factorial design, the factors were four crude protein levels: (18, 21, 24 and 27%) and four digestible energy levels: (3,000; 3,300; 3,600 and 3,900 kcal/kg). Nitrogen retention studies were conducted during the 4th week after the animals were 4 weeks on test diets. There were 6 pigs (3 females and 3 males) in the nitrogen balance trials. Apparent protein digestibility was calculated from the faecal collection. Urinary urea, citrulline and allantoin were also determined. Six out of the eight experimental pigs per diet were selected for bleeding and the plasma urea concentrations were determined. There was a significant (0.05) Protein x Energy, Energy x Sex and Protein x Energy x Sex interactions. Females retained significantly higher proportions of their nitrogen intake than the castrated males, the lowest nitrogen retention was obtained on the lowest protein and lowest energy combination. The best nitrogen retention in males was obtained on the 21% protein diet with 3,600 kcal DE/kg, while in females; the best was obtained on the diet containing 24% protein. Total urinary urea concentration was significantly (p 0.01) influenced by both protein and energy levels and significant Protein x Energy interactions also occurred. Dietary protein, expectedly, significantly influenced plasma urea concentrations tending to be lowest on the two intermediate energy levels; 3,300 and 3,600 kcal DE /kg. Nitrogen retention was optimum between 18 and 21% protein levels and castrated males tended to show optimum nitrogen economy on lower protein levels than female pigs. Total urea concentration paralleled nitrogen retention profile. The Kiriya Index (A/U x IP) may be a precise method to determine protein requirement for pigs in the humid tropical environment. Digestible energy levels of between 3,300 and 3,600 kcal/kg would be adequate for maximum nitrogen economy.

Keywords: crude protein, digestible energy, nitrogen retention, urinary, blood constituents.

Introduction

Nitrogen retention has been demonstrated to be directly influenced by the level of protein intake (Oyeleke, 1985) and by energy intake and dietary protein concentration (Campbell and Dunkin, 1983). Urinary nitrogenous components and the index, A/u x PI (A = Allantoin; U = Urea and PI = Protein Intake), have been adjudged to be reliable assessments of dietary protein and/or amino

acid adequacy in pigs and laboratory animals (Balogun and Fetuga, 1981, Balogun and Fetuga, 1982, Balogun *et al*, 1984, Oyeleke *et al* 1985). Nitrogen retention studies have been shown to be an accurate parameter to assess the effects of metabolic potentials and efficiency of nutrients, protein and energy (when other essential nutrients are adequate) on the desired production targets, especially, the lean, which commands high prices and also

adjudged to meet nutritional requirements for most economic production and healthy living in humans (Oyeleke *et al*, 1985).

Knowledge of food and nutrition has confirmed superiority of animal protein over other sources of essential amino acids. Several studies have convincingly demonstrated that nitrogen retention in pigs is directly influenced by the level of protein intake (Campbell and Dunkin, 1974, Oyeleke *et al* 1987) and protein and energy intake (Cooke *et al*, 1982, Black, 1974). The links among nitrogen retention and nitrogen intake, nitrogen retention and energy intake have been well articulated (Black 1974). The fact that protein and energy intake resulted in different responses to nitrogen retention in growing animals suggested an interdependence between protein and energy levels in determining how nitrogen is metabolized at different growth phases of animals. A biphasic response in growing finishing pigs has been demonstrated (McCracken *et al* 1980). These findings suggested further that there are two phases in the response of protein and energy intakes to nitrogen retention in a growing pig.

This paper, aims at investigating how widely varying crude protein and energy levels can influence nitrogen retention in growing pigs raised from 9 and 60kg, a period assumed to cover the two phases (protein dependent and energy dependent). Earlier studies examined few protein and energy levels. This paper employed a much wider range believed to offer a better and deeper understanding of the possible effects of varying protein and energy levels on nitrogen retention and metabolic constituents in growing/finishing pigs in a tropical humid environment.

Materials and methods

Animals

A total of one hundred and twenty-eight Large White x Landrace pigs weighing initially about 9.0 ± 1.5 kg were assigned to sixteen groups of eight pigs each, comprising four females and four barrows. Allocation to groups was on the basis of sex, initial weight and as far as possible litter origin, such as to equalize group mean weight and sex. These groups were then randomly assigned to any one of the sixteen treatments laid out in a 4x4 factorial design. The factors were four crude protein levels and four digestible energy levels.

Experiment diets

The composition of the experimental diet and the basic ingredients used are as presented in Table 1. There were sixteen diets, in all, consisting of four digestible energy levels 3,000; 3,300; 3,600 and 3,900 kcal/kg and four crude protein levels 18, 21, 24 and 27% at each dietary energy level. Attempts were made to maintain approximately the same ratio of the main protein contributing ingredients, fish meal, blood meal, groundnut cake, and palm kernel meal. This was to ensure that approximately equal proportions the amino acids were supplied from animal and plant sources and to ensure similar ratios of amino acids to protein. Synthetic DL-methionine, L-Lysine hydrochloride were added to maintain a constant balance between the level of amino acids relative to the dietary protein levels. The energy levels used were obtained by appropriate adjustment of the level of yellow maize, cassava flour, wheat offal's and brewers dried grains, while the higher energy diets were arrived at by the inclusion of varying levels of palm oil, highly digestible oil. Dietary calcium and phosphorus levels were equalized by the inclusion of di-calcium phosphate. Constant levels of

Table 1: Diet composition % dry matter.

Ingredient	Diets															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ground Yellow maize (10% cp) ¹	32.4	31	30	30	43	41	38	37	49	45.57	41.7	38.21	52.2	48.46	43.96	40.42
Cassava flour (1.3% cp)	13.17	11.25	8.96	5.07	10.68	8.43	6.64	3.14	7.11	5.31	3.39	0.39	4.7	1.9	-	-
Wheat offals (22.3% cp)	20	15.16	11.02	7.21	13.2	10.3	7.27	4.76	8.71	6.8	4.8	3.14	5.75	4.49	3.17	-
Fried Brewers grains (16.3% cp)	10	10.41	11.02	10.81	3.3	3.44	3.64	3.57	1.69	1.14	1.2	1.18	0.36	0.38	0.4	-
Palm kernel meal (18.5% cp)	7.57	10	12.43	14.86	8.49	11.03	13.84	16.43	8.87	11.41	14.22	16.81	9.03	11.57	14.33	16.92
Groundnut Meal (19.0% cp)	4.59	6.5	8.41	10.32	5.47	7.14	8.96	10.63	5.71	7.38	9.2	10.89	5.81	7.48	9.28	10.95
Fish Meal (65% cp)	2.6	3.73	4.79	5.85	3.09	4.05	5.06	6	3.23	4.19	5.2	6.14	3.29	4.24	5.25	6.19
Blood Meal (80% cp)	5.55	7.25	9.15	11.03	6.15	8.03	10.06	11.93	6.33	8.32	10.35	12.22	6.57	8.42	10.44	12.31
Palm oil	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Dicalcium Phosphate	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Vitamin-Mineral-Premix ²	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Common salt	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm	100ppm
Zinc oxide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anticidant	0.2	0.18	0.16	0.08	0.17	0.14	0.13	0.01	0.16	0.15	0.12	0.07	0.17	0.14	0.11	0.07
L-Lysine %	-	0.03	0.04	0.07	0.05	0.07	0.1	0.11	0.07	0.08	0.11	0.14	0.08	0.09	0.11	0.16
DL-methionine %	-	0.01	0.02	0.03	0.02	0.03	0.04	0.04	0.03	0.03	0.05	0.05	0.03	0.04	0.05	0.06
L-Tryptophan %	18	21	24	27	18	21	24	28	18	21	24	27	18	21	24	27
Calculated Crude Protein %	3000	3300	3600	3900	3000	3300	3600	3900	3000	3300	3600	3900	3000	3300	3600	3900
Calculated digestible energy Kcal/kg	16.67	14.29	12.5	11.11	18.33	15.71	13.75	12.22	20	17.14	15	13.33	21.67	18.57	16.25	14.44
Calculated caloric-protein ratio																

¹CP = Crude protein of ingredient as determined by Oyeleke (1988) and Fajana (1972)² Composition of Vitamin-mineral-Premix: (A) Pfizer (Nig.) Ltd. Product) was as follows:Vitamin A 6,000,000 IU; Vitamin D₃ 800,000 IU; Vitamin E (Biotin) 4,000 mg; Panthoic acid 14,900 mg;Vitamin B₂ 20mg; Vitamin B₆ Nicotinic acid 50,000mg; Choline chloride 500,000mg; Zinc Basitron 10,000mg;

Antioxidant 125,000mg; Iron (Fe) 100,000mg; Manganese (Mn) 60,000mg; Zinc (Zn) 125,000mg; Copper 75,000mg;

Cobalt (Co) 500mg; Iodine (I) 2,000mg.

sodium chloride were included in all diets, while zinc oxide was included in the diets to supply additional 100ppm zinc. A commercial vitamin-trace mineral premix were supplied by a commercial Vitamin-Mineral Premix (Pfizer (Nig.) Ltd Product). All experimental diets were analyzed at each mixing and at regular intervals during the course of the experiment for their proximate constituents (A.O. A.C, 2005). The average values of the diets are presented in Table 2. Digestible energy values were determined using standard procedures (Fetuga *et al*, 1977). Good agreements were found between calculated and analyzed values showing the analyses of the raw materials used in the calculations were reliable. The amino acid content of each diet (as presented in Table 3) was calculated from amino acid analysis of the ingredients obtained on several batches using a Technicon (TSM) amino acid analyzer (Fetuga *et al* , 1973) and the values expressed as percentage of protein are given in Table 4.

Housing and Feeding

Pigs were housed in concrete floored pens, each with 8 individual feeding units. The pigs were assigned numbers on their back and on the corresponding feeding unit buckets, such that the number on the pigs tallied with the number on the bucket and diet to which the animal was allocated.

Each adjacent pen housed 8 animals and each pig had a feeding record to facilitate keeping of records. Feeding troughs were provided in the individual feeding units. The animals were fed three times daily at 07.30 hours, 12.30 hours and 16.30 hours when they were led into the individual feeding stalls and allowed to eat all they could within 90 minutes. After eating they were allowed into adjacent play pens where fresh water was made available *ad libitum*. Water was changed four times daily, since

the pigs wallowed occasionally.

Management

It has been reported that sound management and good husbandry practices , apart from encouraging good growth it also ensures that the debilitating effects of diseases and parasites are averted and ,most importantly, ensure that responses were mainly due to dietary treatments.

The animals were therefore subjected to routine de -worming monthly, immediately after the weekly weighing with 'Piperazine^R Citrate'. The inner and outer pens were disinfected with non-poisonous 'Izal^R', every morning after thoroughly washing the pens.

Records

Pigs were weighed weekly and weekly feeding consumption was also recorded. On the last day preceding the weighing day, all feed left in the troughs were collected and weighed for the individual pigs. The amount so collected was subtracted from total weighed in the week to obtain accurate weekly consumption.

Nitrogen Balance Trials

Nitrogen balances were conducted during the 4th week after the animals were a full 4 weeks on the test diets and were assumed to have sufficiently adjusted to the experimental diets and also during the 10th week on the experimental diets.

There were 6 animals per treatment, 3 gilts and 3 females per treatment, put in special metabolism cages (Oyenuga, 1961) which affords the animal's relative comfort and enables separate collection of urine and faeces. Feed and water were made available *ad libitum*. On all occasion when pigs were transferred from the pens to metabolism cages, they were allowed a three day adjustment period to get accustomed to the cages before collection was started. Faecal and urinary collection lasted five days. Chromic oxide was used as a marker at the

beginning and at the end of the trial. Faeces and urine were collected daily, weighed and measured respectively. About 2mls of concentrated sulphuric acid (H_2SO_4) was used to rinse the buckets for urine collection to prevent frothing and ammonia production which might result in nitrogen losses.

1% of each daily faecal and urine collection were taken after mixing and shaking the daily collection thoroughly. Urine was collected into urine bottles to which a thin layer of toluene had been added. Each day's collection was kept refrigerated at $-5^\circ C$ till the end of the collection when the samples for pig were pooled and stored in deep freezer at $-5^\circ C$ until analyzed. 2% of daily faecal collection was also pooled and stored in polythene bags at $-5^\circ C$. On the sixth day the pooled sample for each animal was thawed, allowed to equilibrate with atmosphere and then dried at $60^\circ C$ for 72 hours. Nitrogen determinations were made on each batch of diets by standard methods (A.O.A.C. 2005). The combustible energy of feeds and dried faeces was determined with a Gallenkamp oxygen ballistic bomb calorimeter using thermo-chemical grade benzoic acid as the standard. All animals were weighed at the commencement and at the end of the collection period while accurate records of feed intake during this period was also kept. From the total feed intake, total dry faeces output and the determined nitrogen content of feed and faeces, it was possible to calculate apparent protein digestibility.

The bulked urinary samples were thawed and filtered through glass wool to remove extraneous particles and 5ml of duplicate samples taken for nitrogen analysis according to Kjeldhal procedure (A.O. A C, 2005). The urinary content per ml and the total volume of urine enabled the computation of total urinary nitrogen

output, which taken together with the faecal nitrogen output enabled the computation of nitrogen retention. The urinary urea, citrulline and allantoin concentration were determined by a standard method (Archibald, 1944) as modified by (Ratner, 1955).

The ratio, $(A/u \times PI)$, allantoin /urea $\times$ protein intake, was estimated and using the protein intake (IP), during the collection period, the index $A/u \times PI$ (Kiriyaama, and Ashida, 1962, Kiriyaama 1970), was calculated.

Dietary and faecal gross energy were determined on duplicate samples of feed, faeces and urine using the Gallenkamp ballistic bomb calorimeter (Gallenkamp & Co. Ltd, London) according to a standard method (Robinson *et al*, 1964). The values in conjunction with total feed intakes and dry faeces output enabled the calculation of digestible energy.

Bleeding and Blood Analysis

Six out of the eight experimental pigs per diet were selected for bleeding. These were bled at the start of the experiment followed by an initial two weekly bleeding up to 25kg and then three weekly between 25 and 45kg. All animals were also bled just prior to slaughter when they attained slaughter weight of approximately 60kg. On each bleeding day, all animals were weighed and fed early in the morning at 5.00 hour and bled 6 hours after the morning feed. About 10ml of blood was withdrawn from the anterior vena cava of each pig with a syringe that had been flushed preciously with sodium citrate. The blood was then carefully transferred to heparinised bottles and stored in an ice bath pending centrifugation, which was done later at $2^\circ C$ for 25 minutes, the plasma separated into plastic vials which were then deep frozen until analyzed. Plasma urea concentration was determined by tested methods (Fawcett

Table 2: Determined nutrient levels in experimental diets (% dry matter)

Diets	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Crude protein %	18.64	21.48	24.48	20.02	18.87	21.72	23.18	27.23	18.14	20.89	24.23	27.51	18.07	21.01	23.69	27.74
Digestible energy kcal/kg	3030	3039	3117	3114	3341	3281	3325	3390	3596	3626	3566	3635	3857	3856	3970	4017
Crude fiber %	7.9	7.04	6.82	6.69	5.98	5.96	5.9	5.97	5.65	6.07	5.28	6.37	5.27	5.31	6.1	5.61
Ether extract %	1.78	2.51	2.35	2.61	2.93	3.639	3.98	4.63	5.35	6.23	6.46	6.06	7.51	7.04	7.76	8.03
Ash %	6.82	6.25	7.86	6.81	5.4	7.31	7.38	8.61	6.42	5	6.8	6.86	5.2	7.53	7.15	6.5
Nitrogen free extract %	64.89	62.96	58.49	56.87	66.82	61.35	58.96	53.56	64.44	61.81	57.23	53.2	53.95	60.51	55.3	52.12

Table 3: Calculated amino acid composition expressed as percent of diet

Amino acids	Diets	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arginine	1.33	11.6	1.88	2.1	1.37	1.61	1.87	2.14	1.39	1.61	1.89	2.15	1.37	1.62	1.89	2.11	
Histidine	0.68	0.83	0.96	1.08	0.78	0.88	1.01	1.15	0.79	0.91	1.16	1.16	0.8	0.91	1.04	1.15	
Isoleucine	0.64	0.64	0.72	0.88	0.66	0.66	0.73	0.81	0.57	0.63	0.72	0.9	0.56	0.63	0.72	0.76	
Leucine	1.83	2.12	2.4	2.69	1.9	2.15	2.45	2.74	1.94	2.19	2.47	2.74	1.98	2.2	2.47	2.71	
Lysine	1.18	1.375	1.575	1.769	1.18	1.375	1.575	1.769	1.18	1.375	1.575	1.769	1.18	1.375	1.575	1.765	
Methionine	0.23	0.29	0.34	0.41	0.26	0.32	0.38	0.43	0.28	0.33	0.39	0.45	0.29	0.34	0.4	0.46	
Cystine	0.26	0.28	0.31	0.32	0.23	0.25	0.27	0.3	0.21	0.24	0.26	0.28	0.2	0.23	0.25	0.27	
Methionine + Cystine	0.49	0.57	0.65	0.73	0.49	0.57	0.65	0.73	0.49	0.57	0.65	0.73	0.49	0.57	0.65	0.73	
Phenylalanine	1.09	1.19	1.37	1.55	1.02	1.19	1.37	1.55	1.09	1.18	1.38	1.59	1.09	1.19	1.37	1.54	
Tyrosine	0.74	0.86	0.96	1.07	0.75	0.85	0.94	1.03	0.74	0.83	0.93	1.04	0.75	0.84	0.93	1.01	
Phenylalanine + Tyrosine	1.74	2.05	2.33	2.62	1.771	2.04	2.31	2.58	1.74	2.01	2.31	2.45	1.75	2.03	2.3	2.66	
Threonine	0.78	0.97	1.05	1.15	0.81	0.93	1.04	1.17	0.81	0.92	1.05	1.18	0.8	0.92	1.05	1.15	
Tryptophan	0.19	0.22	0.255	0.285	0.19	0.22	0.255	0.285	0.19	0.22	0.255	0.285	0.19	0.22	0.25	0.285	
Valine	1.03	0.72	1.49	1.7	1.07	1.39	1.51	1.73	1.1	0.77	1.51	1.74	1.12	1.33	1.52	1.72	

and Scott, 1960, Eggum, 1970).

Statistical analyses

The data from the experiment were subjected to the multi-factor analysis of variance and where significant differences were indicated means were compared using the Duncan's new multiple range test (Steel and Torrie, 1980). Regression analyses were also carried out on some parameter. Protein levels and intakes and energy levels and intakes were regressed separately against the selected parameters. Step wise regression analyses were also carried with both protein and energy intakes as independent variables to evaluate the relative contribution of these to the observed differences. All of this statistical analysis was achieved using the SPSS Program on the IBM computer.

Results

Nitrogen Retention

Table 5 gives the summary of nitrogen retention. There were significantly ($P < 0.001$) main treatment effects on nitrogen retained as percentage of intake. There were significantly ($P < 0.001$) protein x energy, energy x sex and protein x energy x sex ($P < 0.01$) interactions, as well as significant sex differences in average nitrogen retention, with the females retaining significant higher proportions of their nitrogen intake than castrated males. In the castrated males, increasing protein levels from 18 to 21% resulted in a significant improvement in percentage of nitrogen intake retained, while increasing protein levels beyond this gave non-significant decline in nitrogen retention. For females and the pooled data, increasing protein levels showed increases in nitrogen retention up to the 24% protein level followed by a decline at the 27% protein diets were however not significantly different. Increasing dietary

energy level also tended to increase nitrogen retention up to 3,600 kcal DE/kg in all cases, after which, an insignificant decline was observed. In all cases within protein level, this trend with regard to energy was observed. The lowest nitrogen retention was obtained on the lowest protein, lowest energy combination. Significantly the best nitrogen retention in males was obtained on the 21% protein diet with 3,600 kcal DE/kg, while in females the best nitrogen retention was obtained at the same energy level but with a diet containing 24% protein. There was, however, no significant difference between this and the immediate lower protein level at the same energy level or the energy level above this at the same protein level.

Urinary Nitrogenous constituents and the index $\{(A/U) \times PI\}$

The effect of varying dietary protein and energy levels on some urinary nitrogenous constituents are summarized in Table 6. Total urinary urea concentration was significantly ($P < 0.001$) influenced by both energy and protein levels. There was a significant energy x protein interaction. Urinary urea concentration was initially high on the 18% protein diet while, significantly, the highest value was obtained on the 27% protein diet. The lowest urinary urea concentration was obtained on the diet with 3,300 kcal DE/kg, although this was not significantly lower than the value on the preceding energy level or the immediate higher energy level. The urinary urea concentration obtained on the highest energy level was, however, significantly higher than for the group fed diets with 3,300 kcal DE/kg. At the 18 and 21% protein levels, minimum urea concentration were encountered when the energy level in the diets was 3,600 kcal DE/kg, while on the 24% protein diet this was with the diet containing 3,300 kcal

Table 4: Amino acid composition of diets as percent of total protein

Amino acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arginine	7.39	7.62	7.83	7.78	7.61	7.67	7.79	7.93	7.72	7.67	7.88	7.96	7.61	7.71	7.88	7.81
Histidine	3.78	3.95	4.00	4.00	4.33	4.19	4.21	4.26	4.39	4.33	4.83	4.30	4.44	4.33	4.33	4.26
Isoleucine	3.56	3.42	3.38	3.26	3.33	3.14	3.04	3.60	3.17	3.00	3.00	3.33	3.11	3.00	3.00	2.81
Leucine	10.17	10.10	10.00	9.96	10.56	10.23	10.21	10.15	10.78	10.43	10.29	10.15	11.00	10.48	10.29	10.03
Lysine	6.55	6.55	5.83	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.56	6.55
Methionine	1.28	1.38	1.42	1.32	1.55	1.52	1.58	1.59	1.56	1.57	1.62	1.67	1.61	1.61	1.67	1.70
Cystine	1.44	1.33	1.29	1.19	1.28	1.19	1.12	1.11	1.16	1.14	1.08	1.04	1.11	1.10	1.04	1.00
Methionine+cystine	2.75	2.71	2.71	2.71	2.73	2.71	2.70	2.70	2.72	2.71	2.70	2.71	2.72	2.71	2.71	2.70
Phenylalanine	5.56	5.67	5.71	5.74	5.67	5.67	5.71	5.74	5.56	5.62	5.75	5.61	5.81	5.56	5.17	5.67
Tyrosine	4.10	4.09	4.00	3.96	4.17	4.05	3.91	3.85	4.11	3.95	3.88	3.85	4.17	4.00	3.88	3.74
phenylalanine+tyrosine	9.66	9.76	9.71	9.70	9.84	9.72	9.62	9.59	9.67	9.57	9.63	9.60	9.73	9.67	9.59	9.41
Threonine	4.30	4.63	4.38	4.26	4.30	4.43	4.33	4.33	4.50	4.38	4.38	4.37	4.44	4.38	4.38	4.26
Tryptophan	1.06	1.05	1.06	1.06	1.06	1.05	1.06	1.06	0.06	1.06	1.05	1.06	1.06	1.06	1.06	1.06
Valine	5.72	6.04	6.21	6.30	6.14	6.29	6.37	6.11	6.11	6.24	6.29	6.44	6.22	6.33	6.33	6.37

Table 5: Influence of varying protein and energy concentration on nitrogen retention in growing pigs

Digestible energy level kcal/kg.	Concentrated Males						Females						Pooled Means					
	Protein levels (%)						Protein levels (%)						Protein levels (%)					
	18	21	24	27	Mean		18	21	24	27	Mean		18	21	24	27	Mean	
3.000	45.64a	59.13b	53.13c	50.37c	52.07c	49.80a	57.35b	60.89bc	63.56cd	58.03c	47.73a	58.49bc	57.18bc	56.47bc	54.72c			
3.300	56.75bc	61.27b	56.80bc	59.35bd	58.54c	54.97cd	57.81b	64.11cd	65.44cd	60.40xy	53.61b	59.27cd	60.43cd	62.40d	59.43y			
3.600	62.15cd	73.71c	65.28cd	60.46cd	65.40c	64.66cd	67.78d	68.97d	61.35c	65.74y	63.40d	70.85f	67.13ef	60.91cd	65.56c			
3.900	61.89cd	60.99bd	63.83bd	63.78bd	62.02y	61.15c	60.53bc	67.57d	56.36b	61.40xy	61.52cd	60.76cd	65.71c	60.07cd	62.02z			
Mean	35.16c	63.76y	59.84xy	58.24xy		57.53x	63.33y	65.39y	61.68xy		57.00x	62.34y	62.61y	59.96xy				
	A.S.E. = 2.49						A.S.E. = 1.61						A.S.E. = 1.84					

+ the values represent nitrogen retained as percentage of nitrogen intake.

Six pigs were used per treatment comprising three females and three concentrated males.

Table 6: Influence of varying protein and energy concentration on some urinary components of growing pigs and the Index ($\frac{A}{U} \times IP$)

		Digestible energy level					
Components		kcal/kg	18	21	24	27	mean
Total urinary	3,000		6.23a	7.37c	7.54c	6.41a	6.89xy
urea (g)	3,000		6.17a	6.41ac	4.15d	7.74ac	6.11x
	3,600		6.07a	3.55d	7.58c	10.62a	6.96xy
	3,900		9.18b	6.68ac	7.05c	7.29c	7.55y
	mean		6.91x	6.00y	6.58y	8.02z	-
		A.S.E. = ± 0.03					
Total Urinary	3,000		258.26ad	244.09ad	178.77a	259.39ad	235.13x
citrulline	3,000		249.95ad	200.09a	200.18a	192.50a	210.85x
(mg)	3,600		174.20a	231.42a	231.60a	237.71a	218.73x
	3,900		351.72b	451.82c	252.65ad	317.45bd	343.41y
	mean		258.53	282.02	215.80	251.76	-
		A.S.E. = ± 30.88					
Total Urinary	3,000		490.14	363.41	450.81	315.01	404.84xy
Allantoin	3,000		217.13	413.47	527.94	562.50	430.26x
(mg)	3,600		488.23	406.56	306.46	452.70	413.50xy
	3,900		375.52	565.26	302.57	331.12	393.62y
	mean		392.76	437.18	396.95	415.33	-
		A.S.E. = ± 17.73					
Kiriyama's	3,000		93.91a	68.49a	87.89a	84.82a	83.78x
index* (A/U x IP)	3,000		87.83a	204.15c	174.21a	81.62a	136.95y
	3,600		81.62a	121.88a	115.42a	46.65b	91.39x
	3,900		43.59b	76.78a	74.32a	90.80a	71.37x
	mean		76.74x	117.83y	112.96xy	75.97x	-
		A.S.E. = ± 14.30					

*A=Allantoin, U=Urea, IP=protein intakes

DE/kg. The least value for urinary urea was obtained with the diet containing 21% protein and 3,600 kcals DE/kg. This observation paralleled the observation regarding optimum nitrogen retention. Energy but not protein levels significantly influenced urinary citrulline levels ($P < 0.01$). Protein X Energy interaction was however significant ($P < 0.01$). No significant differences were found for the average citrulline level on the three lower energy levels, while the average value at the lowest

energy level was significantly higher. At the 18% dietary protein level, urinary citrulline concentration decreased non-significantly with increasing energy level reaching a minimum value on the 3,600 kcals DE/kg level and then rising steeply at the highest energy level. At the three higher protein levels, minimum citrulline concentration occurred on the diets with 3,300 kcals DE/kg. Even though protein level did not significantly influence urinary citrulline level, a minimum value was obtained on the 24% protein diet.

Urinary allantoin concentration was not significantly or consistently affected by protein level whereas dietary energy level significantly ($P < 0.01$) influenced the concentration of this metabolite. No significant protein $\times$ energy interactions occurred. Allantoin concentration increased when energy level was increased from 3,000 kcal DE/kg to 3,300 kcal DE/kg and then decreased progressively at higher energy levels, the value at 3,300 kcal DE/kg being significantly higher than only the value on the highest energy level. The index, $\{(A/U) \times PI\}$, was significantly influenced by both energy ($P < 0.01$) and protein levels. There was also a significant protein $\times$ energy interaction. Increasing dietary protein level from 18 to 21% resulted in a sharp rise in the index, declining non-significantly at the 24%

protein level and significantly on the 27% protein diet. A sharp rise in the index was also obtained when energy level was raised from 3,000 to 3,300 kcal DE/kg. The index dropped significantly thereafter. The highest value for this index was obtained in the group fed the diets containing a combination of 21% protein and 3,300 kcal DE/kg.

Plasma urea concentration

The plasma urea concentrations in pigs fed varying dietary protein and energy concentrations taken during six different stages of growth from 9 to 60 kg liveweight are presented in Table 7.

There were no significant interactions between main effects; therefore the responses to protein and energy are presented separately. There were however sex $\times$ diet interactions and so values are

Table 7: Influence of varying dietary protein and energy concentration on total plasma urea (mg/100ml) of pigs reared between 9 and 60 $\pm$ 1.5 kg liveweight

	Sex	Crude Protein Level (%)				Crude Protein Level (Kcal/Kg)				Sex	A.S.E.
		18	21	24	27	3,000	3,300	3,600	3,900	Means	$\pm$
Period I	Cm	24.26	24.63	25.33	24.98	24.65	24.69	23.72	26.14	24.8	0.43
	F	20.03	20.69	22.51	22.59	21	20.42	19.93	24.46	21.46	0.64
	Both	22.19	22.66	23.9	23.75	22.82	22.6	21.8	25.29	-	0.58
Period II	Cm	26.77a	21.72b	22.97b	26.07a	28.83a	24.52b	25.23b	28.94a	24.62	0.61
	F	19.89	19.12	21.71	20.08	21.66	20.09	18.27	20.78	20.2	0.52
	Both	24.97ac	20.42b	21.69bc	23.93c	25.25	22.36	23.32	24.86	-	0.54
period III	Cm	18.47	17.81	15.93	17.15	15.9	16.8	16.25	16.4	17.34	0.89
	F	18.56a	16.72a	13.60b	14.30b	15.03	14.84	14.08	14.23	15.77	0.76
	Both	18.52a	17.27a	14.77b	15.72ab	15.47	15.83	15.16	15.31	-	0.82
Period IV	Cm	15.31	15.7	14.65	18.1	16.71	15.74	15.96	15.37	15.94	1.16
	F	13.7	13.69	13.21	15.48	14.13	14.31	14.09	13.55	14.02	0.98
	Both	14.5	14.69	13.93	16.75	15.42	14.98	15.02	14.46	-	1.02
Period V	Cm	21.52	20.33	20.37	21.59	20.78	20.89	19.02	23.12	20.95	1.64
	F	17.46	17.1	18.16	18.11	17.98	15.4	18.08	19.37	17.71	1.52
	Both	19.96	18.71	19.27	19.85	19.38	18.61	18.55	21.24	-	1.46
Period VI	Cm	25.56	27.3	23.58	24.09	27.98	23.42	23.53	25.6	25.13	1.82
	F	23.94	21.33	22.92	23.71	23.98	22.46	21.92	24.36	23	2.11
	Both	24.85	24.31	23.24	26.4	25.98	22.46	22.84	24.98	-	1.94

presented for the different sexes. Dietary protein levels significantly influenced for plasma urea concentrations (P 0.05) only during the second and third bleeding periods, while energy concentration significantly influenced (P 0.05) this metabolite during the second bleeding period only. In the second bleeding period the means for the castrated male and the pooled data gave the lowest plasma urea concentration on the diet containing 21% crude protein. This value was significantly lower than those obtained on the lowest and highest dietary protein level but not the 24% protein diet. Also during this period the intermediate energy levels (3,300 and 3,600 kcals DE/kg) gave plasma urea values significantly lower than those observed on the lowest and highest digestible energy level. In period three, only the female means with respect to protein were significantly different. Increasing dietary protein level resulted in decreasing plasma urea concentration reaching a minimum value on the 24% protein diet and then rising non-significantly when protein level was increased to 27%. Protein levels did not influence plasma urea concentration in period I, IV and VI, although during these periods minimum concentrations were found between the 21 and 24% dietary protein levels, the values on the lowest and highest protein levels being usually higher. The same trend was observed with respect to energy, that plasma urea concentration tended to be lowest on the two intermediate energy levels (3,300 and 3,600 kcals DE/kg) being higher on the lowest and highest energy levels during most of the period. The differences between sex means were significant during period I and II but not during the subsequent periods.

Discussion

In this experiment, there was a significant interaction between protein and energy in terms of nitrogen retention. Optimum nitrogen retention in castrated males was obtained when the diet contained 21% crude protein and 3,600 kcals DE/kg while in females this occurred at the same energy value. The lowest nitrogen retention was encountered on the diet with the lowest protein and energy level combination. The levels of energy and protein for optimum nitrogen retention were close to those earlier recommended (N.R.C, 1973, Campbell and Dunkin, 1974) and Cromwell *et al*, 1978)]. Low nitrogen retention was also found when low protein, low energy diets were fed. These researchers reported that increasing supplemental fat from 2 to 12% and protein levels from 12 to 16% gave significantly higher nitrogen retention. A report (Black 1974) found that nitrogen retention showed no significant relationship with digestible energy concentration of the diet, suggesting that pigs on higher energy diets did not benefit to the same degree expected from the greater amount of nitrogen digested by them. Discrepancies regarding utilization of dietary energy for nitrogen retention particular when expressed relative to the total dietary nitrogen consumed may arise mainly from differences in the composition. Low energy diets are achieved by inclusion of fibrous materials while high energy levels in diets are achieved by the inclusion of less of high fibre ingredients and more of highly digestible ingredients and sometimes fat. Digestibility of the protein associated with the high fibre diets (low energy) may be impaired and therefore amount of nitrogen absorbed and retained reduced. Apart from the effect of fibre *per se* in protecting protein from proteolytic enzyme breakdown, low energy diets achieved by inclusion of fiber or diluents

may affect the rate of passage of the ingesta and thus impair protein digestibility of even the energy yielding components such as carbohydrates. Kiriya and Asida, (1964) reported that the inclusion of diluents in diets reduced the digestibility of the protein and carbohydrate in the diet, while later investigation of Campbell and Dunkin (1983) found that digestibility of nitrogen was lower at low digestible energy levels and increased with increasing digestion energy concentration of the diet. That energy was the major factor influencing nitrogen retention in this study, was borne out by the stepwise regression analysis which indicated that 45.69% ($r = 0.67$) of the total variability (46.55%) was due to energy intake and only 0.86% ($r = 0.14$) was due to dietary protein intake. The trends observed for nitrogen retention was further strengthened by a more detailed analyses of the urinary components. Total urinary citrulline and allantoin indicated from the significant protein x energy interaction that higher energy levels are required to maximize the utilization of high intakes of dietary protein (Oyeleke *et al*, 1985). The lowest value of urinary urea indicated point of most efficient protein utilization was obtained on the diet containing 21% protein and 3,600kcal DE/kg. Urinary allantoin and citrulline suggested protein levels of 18% with an energy level of 3,600 kcal DE/kg for castrated males while these components indicated that a diet with 24% protein and 3,600 kcal DE/kg gave optimum nitrogen economy in females.

The index, $\{(A/U) \times PI\}$, which has been shown to be a sensitive indicator of protein quality and efficiency of feed utilization (Kiriya and Ashida, 1962 and 1964), showed a requirement combining 21%protein level with 3,300 kcal DE/kg. All of these values tended to revolve around

the combination indicated by nitrogen retention and may offer no advantage over the combination indicated by nitrogen retention and may offer no advantage over the determination of total urinary nitrogen, in a carefully conducted balance experiment. It is however important to stress that the balance experiments were conducted during the early growth phase of the pigs when requirements for nitrogen are known to be initially high and this may account for the slightly higher requirements for the male compared to that indicate by growth rate and feed efficiency.

Plasma urea concentrations indicated a dietary energy need between 3,300 and 3,600 kcal DE/kg throughout most of the breeding periods covered and a protein need of between 21 and 24% in the first four bleeding periods decreasing to 18% in the 5th and 6th period when the animals had attained much heavier weights indicating the sensitivity of this parameter to changing protein requirements with advancing age.

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