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Dietary Modelling of Nutrient Densities: Effect and Response in Different Growing Phases on Growth Performance, Nutrient Digestibility, Litter Quality and Leg Health in Turkey Production

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ABSTRACT

An experiment was conducted to explore the time bound (different growth phases) effect of different dietary nutrient densities i.e., different energy and protein concentration while maintaining the ratio between the two, all with the same ideal amino acid profile, on litter quality and leg health (footpad dermatitis (FPD) and hock burn (HB)), when fed to growing turkeys. The effects of dietary nutrient modelling on growth performance parameters, water intake and excretion, dry matter (DMD), organic matter (OMD), crude protein (CPD) digestibility coefficients and apparent metabolisable energy (AME) were also examined, when fed to growing turkeys in varying growth phases. At twenty-eight days of age one hundred and seventy five male turkeys (BUT 8) were transferred to 35 floor pens, using stratified randomisation on body weight, 5 birds in a pen, all pens were equipped with plastic feed hoppers and drinkers. The experiment was a randomized block design consisting of 5 treatments (5 levels of CP and ME concentrations and 4 feeding/ growth phases). Each dietary treatment was replicated 7 times with 5 birds in each replicate. Feed and water were offered *ad libitum* throughout the experiment. Five dietary treatments, containing either 77, 85, 100, 110 or 120% of the crude protein (CP) and metabolisable energy (ME) content recommended by the breed standard. The whole experimental period of 16 weeks starting from 4 weeks of age was divided into 4 weeks standard growth phases: 4-8, 8-12, 12-16 and 16-20 weeks, finishing at 20 weeks of turkey's age, according to commercial management guide for BUT 8 (Aviagen Turkeys Ltd.). Nutrient density had a positive and linear effect ($P<0.001$) on weight gain, feed efficiency and dry matter digestibility (DMD) whereas the effect of nutrient density on dietary protein digestibility (CPD) only approached significance ($P=0.081$). As might be expected increasing nutrient density had a negative and linear effect on feed ($P<0.001$) and water ($P<0.01$) intake and did not affect the ratio between these two parameters. Increasing nutrient density had a positive effect on litter quality (linear ($P<0.001$)), with both the litter moisture ($P<0.01$) and the litter score decreasing ($P<0.001$). Conversely litter ammonia concentration increased ($P<0.001$) as nutrient density increased, similarly as nutrient density increased so did the prevalence of hock burn ($P<0.01$). Notably there was no effect ($P>0.05$) of treatment on FPD. The results suggest that an increase in nutrient concentration can reduce the moisture content of the litter and so improve overall litter quality. However, the incidence of hock burn increased with the high nutrient density diets, suggesting that factors other than the litter moisture alone may contribute the occurrence of leg health problems in turkey production.

Key words: Nutrient density, Digestibility, Performance, Wet litter, Ammonia, Footpad dermatitis, Hock burn.

INTRODUCTION

Litter quality is an important component of many poultry production systems but especially for broilers and meat producing turkeys as these birds stay in contact with the litter throughout their life (Ekstrand *et al.*, 1997). High litter moisture and ammonia (NH_3), content and quality are correlated with dirty footpads, footpad dermatitis (FPD) and hock burn (HB) lesions in poultry (Ekstrand *et al.*, 1997; Dawkins *et al.*, 2004; Haslam *et al.*, 2006 and Mayne *et al.*, 2007). Therefore, the three most important aspects of litter quality are the moisture content, stickiness and nitrogen or NH_3 content in the litter (Lister, 2009). A good quality litter should satisfy the bird's welfare requirements by absorbing moisture, providing a warm and dry surface to rest on, providing a substrate that allows microbial activity to degrade excreta and should encourage dust bathing and litter directed activity.

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The effect of dietary energy on feed intake is emphasised in literature which is correlated with water intake. Some reports (Collin *et al.*, 2003) suggest that achieving a higher AME to CP ratio by using a lower CP concentration might encourage birds to increase feed intake to meet their amino acid requirements, which may also increase water intake (WI) and have an impact on the litter quality. However, it is not clear whether the absolute protein concentration itself or the ratio between the dietary protein and energy was the reason for the deterioration of the litter quality or to the changes in the CP to AME ratio. Therefore, the aim of this experiment was to compare the effect on WI and litter quality (e.g. moisture content, pH and NH_3 content) of different nutrient density diets formulated to give a constant CP to AME ratio in all diets and to establish how these dietary modifications can affect litter characteristics and the correlation of these characteristics with the FPD and HB in turkeys.

Materials and Methods

Animal ethics

The study was approved by The Animal Experimental Committee of Scottish agricultural college.

House preparation

Prior to the reception of poults the house was vacant and thoroughly cleaned. This included proper washing and disinfection of the room. A foot dipping tank was in place at all times on the door step of the house to maintain biosecurity.

Feed preparation

In the pre-study period, from 0 to 4 weeks of age, the birds were fed a standard crumb starter turkey feed (table 1). The starter diet consisted of major feed ingredients such as wheat, soybean meal, and fish meal containing crude protein 263 g/kg and ME 12.15 MJ/kg.

Five experimental diets in total were used for each growth phase (4 weeks each and starting at 4 weeks of age until 20 weeks) in the study. The wheat-soybean based diets in pelleted form was prepared according to the formulation for BUT 8 (Aviagen Turkeys Ltd., UK) as presented in table 3 to table 6. Diet T3 served as control with 100% of crude protein and energy according to BUT 8 requirement for each growth phase, while diets T1, T2, T4 and T5 contained 77, 85, 110 and 120% concentration of crude protein and energy, respectively. All the diets were formulated according to the respective growth phase nutrient recommendation of BUT 8 other than protein and energy content. Digestible amino acid profile was similar during a growth phase of 4 weeks for all the diets according to BUT 8 recommendations with some missing data values for amino acids being obtained from Firman and Boling (1998) and upgraded according to commercial values (table 2). Amino acids like lysine, methionine and threonine were included where deficient, to meet the requirement. Each experimental diet for the respective growth phase was fed randomly to selected seven replicates for the period from 4 to 20 weeks. All feed was pelleted. The diets used for experiment were analysed for their dry matter (DM), crude protein (CP) minerals, crude fat (EE), Neutral detergent fibre (NDF), ash, ME and amino acid content.

Dry matter (DM) in feed and excreta was determined by drying at 100°C for 24 hours in a force draft oven (AOAC 925.10, 1990). The fat content was determined with AOAC 920.39 method using a Soxtec 1043 extraction unit (Foss Ltd, Wigan, UK). The dietary neutral detergent fibre (NDF) fraction was determined according to procedure described by Holst (1973).

Feed conversion efficiency, organic matter efficiency and protein efficiency ratios calculations

The Feed Conversion Efficiency (FCE) was calculated by dividing weight gain by feed intake. The same applied for the Organic Matter Efficiency (OME), and for the protein efficiency ratio (PER)-by calculating by dividing body weight gain with total protein intake. Whereas Energy Efficiency Ratio (EER) was calculated as weight gain (kg/d) / AME intake (MJ/d).

Nutrient digestibility coefficients calculations

To determine dietary nutrient digestibility and AME at 7 weeks of age, all the birds from each pen were transferred to one of the 35 raised floor pens for 24 hours. The excreta voided were collected on trays placed beneath each raised floor pen and the feed intake for the same period was determined. Then excreta samples were freeze dried, weighed and milled to pass through a 0.75 mm mesh.

Dietary N – corrected apparent metabolisable energy (AMEn) was determined as previously described (Hill and Anderson, 1958). The coefficients of apparent digestibility of dietary dry matter (DMD), organic matter (OMD) and crude protein (CPD) as well as amino acid digestibility coefficients were also determined by the difference between nutrient intake (feed intake multiplied by the nutrient content in feed) and nutrient output (excreta voided for 24 hours multiplied by the nutrient content in excreta) divided by the nutrient intake.

Comparison of turkey growth performance

One hundred and eighty five day old male turkeys (BUT 8) were weighed and placed in a controlled environment building. For the pre-study period (first 4 weeks of age) birds were placed in the floor pen containing 10 cm thick bedding material of wood shaving. During the pre-study period all birds were offered the same standard turkey starter crumb diet and had *ad libitum* access to feed and water. Birds were wing tagged at day 10 for identification. The average air temperature of the house was recorded every day and was maintained at 30°C for 7 days and gradually reduced to 22°C at 4 weeks of age. For the first day 24 hour light was provided which then changed to a lighting schedule of 16 hour light and 8 hour dark period throughout the trial.

At twenty-eight days of age one hundred and seventy five turkeys were transferred to 35 floor pens, using stratified randomisation on body weight, 5 birds

in a pen (1.01 x 0.35 m/pen floor area) within a controlled environment room. All the pens were equipped with plastic feed hoppers and drinkers. The experiment was a randomized block design consisting of 5 treatments (5 levels of CP and ME concentrations and 4 feeding/ growth phases). Each dietary treatment was replicated 7 times with 5 birds in each replicate. Feed and water were offered *ad libitum* throughout the experiment. The whole experimental period of 16 weeks starting from 4 weeks of age was divided into 4 weeks standard growth phases: 4-8, 8-12, 12-16 and 16-20 weeks, finish at 20 weeks of turkey's age, according to commercial management guide for BUT 8 (Aviagen Turkeys Ltd.). The same house environment as for the end of the pre-study period was provided until the end of the study. The experiment ended when the birds were 20 weeks of age.

Table 1. Ingredient composition (g/kg) of the starter diet fed to the turkeys during the pre-study period from 0 to 4 weeks of age.

Ingredients	g/kg
Fish meal - (72%-CP)	30
Soybean meal - (48%-CP)	275
Wheat	575
Soy oil	17.4
Corn gluten - (60%-CP)	20
Casein	30
Lysine HCl	1.9
DL Methionine	2.8
L-Threonine	3.9
Salt	2.2
Limestone	7
Dicalcium phosphate	21.5
Vit./min. premix[1]	2.8
Coccidiostat	0.5
Pellet binder	10
Calculated nutrient analysis	
Metabolisable energy (ME), MJ/kg[2]	12.15
Crude protein (CP) (g/kg)	263.1
Crude fibre (g/kg)	29
Ca (g/kg)	10
Available Phosphorus (g/kg)	5
Na (g/kg)	1.5
Cl (g/kg)	2.3
K (g/kg)	8.2
Indispensable amino acids	
Arginine (g/kg)[3]	12.2
Cystine (g/kg)[3]	4.2
Isoleucine (g/kg)[3]	9.6
Lysine (g/kg)[3]	13.1
Methionine (g/kg)[3]	5.1
Phenylalanine (g/kg)[3]	10.5
Threonine (g/kg)[3]	8.1
Tryptophan (g/kg)[3]	3.1
Valine (g/kg)[3]	10.4
Dispensable	
Tyrosine (g/kg)[3]	9.4

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diet): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME value of the diet was calculated using the ME values of the dietary ingredients (NRC, 1994).[3]Concentration of amino acid on digestible basis.

Table 2. Ideal protein ratios for different growth phases of turkeys.

Amino acids[3]	Ideal protein ratios expressed as % relative to lysine for different growth phases			
	week 4-8	week 8-12	week 12-16	week 16-20
Arginine[1]	97.5	91.1	90.4	90.3
Cystine[1]	31.6	34.8	34.9	38.7
Isoleucine[2]	71.5	71.1	74.3	78.5
Lysine[1]	100.0	100.0	100.0	100.0
Methionine[1]	38.6	40.7	44.4	45.2
Phenylalanine[2]	78.5	77.8	76.6	74.9
Threonine[1]	61.4	60.0	60.1	60.2
Valine[2]	77.8	77.8	72.2	70.1
Tryptophan[1]	24.1	23.0	22.8	22.6
Tyrosine[2]	70.3	69.6	68.7	66.3

[1]From Aviagen Turkeys Ltd., UK; [2]From Firman and Boling (1998); [3]The ratios between amino acids were calculated on the basis of digestible concentration of each amino acid.

Table 3. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 4-8 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%-CP)	0.00	9.50	27.00	38.50	50.00
Soybean Meal - (48%-CP)	193.0	229.7	297.3	341.8	386.2
Wheat, White	449.6	426.8	384.8	357.2	329.6
Wheat Middlings	150.00	121.50	69.00	34.50	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Corn gluten meal - (60%-CP)	0.00	1.90	5.40	7.70	10.00
Casein	0.00	9.50	27.00	38.50	50.00
Soybean OiL	0.00	23.85	67.77	96.64	125.50
L-Lysine HCl	3.40	2.75	1.56	0.78	0.00
DL-Methionine	2.50	2.75	3.20	3.50	3.80
L-Threonine	3.30	3.64	4.27	4.69	5.10
Common Salt	2.30	2.28	2.25	2.22	2.20
Limestone	12.20	10.72	7.99	6.19	4.40
Dicalcium phosphate	20.00	19.91	19.73	19.62	19.50
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	9.72	10.61	12.26	13.35	14.43
Crude protein (g/kg)	201.4	222.4	261.1	286.6	312.0
Crude fibre (g/kg)	54.30	48.92	39.02	32.51	26.00
Ca (g/kg)	10.00	9.98	9.95	9.92	9.90
Available Phosphorus (g/kg)	5.00	5.00	5.00	5.00	5.00
Na (g/kg)	1.50	1.50	1.50	1.50	1.50
Cl (g/kg)	2.50	2.41	2.23	2.12	2.00
K (g/kg)	8.90	9.01	9.22	9.36	9.50
Mn (mg/kg)	105.7	100.4	90.5	84.0	77.5
Zn (mg/kg)	105.0	99.9	90.5	84.3	78.1
Indispensable amino acids					
Arginine (g/kg)[3]	10.10	11.13	13.02	14.26	15.50
Cystine (g/kg)[3]	3.20	3.54	4.17	4.59	5.00
Isoleucine (g/kg)[3]	6.70	7.65	9.40	10.55	11.70
Lysine (g/kg)[3]	10.20	11.28	13.28	14.59	15.90
Methionine (g/kg)[3]	3.90	4.32	5.09	5.59	6.10
Phenylalanine (g/kg)[3]	7.10	8.13	10.02	11.26	12.50
Threonine (g/kg)[3]	6.20	6.87	8.09	8.90	9.70
Tryptophan (g/kg)[3]	2.50	2.75	3.20	3.50	3.80
Valine (g/kg)[3]	7.30	8.38	10.38	11.69	13.00
Dispensable					
Tyrosine (g/kg)[3]	6.20	7.17	8.95	10.13	11.30

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).[3]Concentration of amino acid on digestible basis.

Table 4. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 8-12 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
			g/kg		
Fish meal - (72%-CP)	0.00	5.70	16.20	23.10	30.00
Soybean Meal - (48%-CP)	80.0	124.7	206.9	261.0	315.0
Wheat, White	510.6	491.8	457.1	434.4	411.6
Wheat Middlings	200.00	162.00	92.00	46.00	0.00
Wheat Bran	150.0	121.5	69.0	34.5	0.00
Corn gluten meal - (60%-CP)	0.00	3.80	10.80	15.40	20.00
Casein	10.00	13.80	20.80	25.40	30.00
Soybean OiL	0.00	27.65	78.57	112.04	145.50
L-Lysine HCl	3.50	3.18	2.58	2.19	1.80
DL-Methionine	2.40	2.69	3.21	3.56	3.90
L-Threonine	1.80	2.31	3.26	3.88	4.50
Common Salt	1.30	1.34	1.41	1.45	1.50
Limestone	10.70	9.71	7.89	6.70	5.50
Dicalcium phosphate	16.00	16.19	16.54	16.77	17.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.04	11.00	12.77	13.94	15.10
Crude protein (g/kg)	169.0	187.2	220.7	242.8	264.8
Crude fibre (g/kg)	50.30	45.63	37.02	31.36	25.70
Ca (g/kg)	8.50	8.50	8.50	8.50	8.50
Available Phosphorus (g/kg)	4.20	4.20	4.20	4.20	4.20
Na (g/kg)	1.20	1.18	1.15	1.12	1.10
Cl (g/kg)	1.90	1.88	1.85	1.82	1.80
K (g/kg)	7.60	7.73	7.98	8.14	8.30
Mn (mg/kg)	106.3	100.4	89.4	82.2	75.0
Zn (mg/kg)	106.9	100.5	88.6	80.8	73.1
Indispensable amino acids					
Arginine (g/kg)[3]	8.10	8.97	10.58	11.64	12.70
Cystine (g/kg)[3]	3.00	3.32	3.92	4.31	4.70
Isoleucine (g/kg)[3]	5.80	6.52	7.85	8.73	9.60
Lysine (g/kg)[3]	8.70	9.63	11.35	12.47	13.60
Methionine (g/kg)[3]	3.60	3.94	4.57	4.99	5.40
Phenylalanine (g/kg)[3]	6.10	6.96	8.53	9.57	10.60
Threonine (g/kg)[3]	5.30	5.87	6.92	7.61	8.30
Tryptophan (g/kg)[3]	2.10	2.31	2.69	2.95	3.20
Valine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Dispensable					
Tyrosine (g/kg)[3]	5.20	6.00	7.47	8.43	9.40

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg. [2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994). [3]Concentration of amino acid on digestible basis.

Table 5. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 12-16 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
			g/kg		
Fish meal - (72%-CP)	0.00	9.50	27.00	38.50	50.00
Soybean Meal - (48%-CP)	41.70	70.83	124.48	159.74	195.00
Wheat, White	614.7	598.5	568.8	549.2	529.6
Wheat Middlings	144.2	116.8	66.3	33.2	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Casein	0.00	7.60	21.60	30.80	40.00
Soybean OiL	0.00	27.1	77.1	109.9	142.7
L-Lysine HCl	4.90	4.37	3.39	2.74	2.10
DL-Methionine	2.80	3.10	3.66	4.03	4.40
L-Threonine	2.10	2.42	3.02	3.41	3.80
Common Salt	1.40	1.38	1.35	1.32	1.30
Limestone	9.00	7.56	4.90	3.15	1.40
Dicalcium phosphate	15.50	15.60	15.77	15.89	16.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.44	11.38	13.12	14.27	15.41
Crude protein (g/kg)	146.5	162.2	191.1	210.0	229.0
Crude fibre (g/kg)	47.70	43.24	35.01	29.61	24.20
Ca (g/kg)	7.50	7.50	7.50	7.50	7.50
Available Phosphorus (g/kg)	3.80	3.80	3.80	3.80	3.80
Na(g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	2.30	2.22	2.08	1.99	1.90
K (g/kg)	6.70	6.66	6.59	6.55	6.50
Mn (mg/kg)	100.4	95.2	85.6	79.3	73.0
Zn (mg/kg)	98.93	93.84	84.45	78.29	72.12
Indispensable amino acids					
Arginine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Cystine (g/kg)[3]	2.80	3.09	3.61	3.96	4.30
Isoleucine (g/kg)[3]	4.70	5.40	6.70	7.55	8.40
Lysine (g/kg)[3]	8.10	8.96	10.53	11.57	12.60
Methionine (g/kg)[3]	3.60	3.98	4.68	5.14	5.60
Phenylalanine (g/kg)[3]	5.00	5.74	7.11	8.00	8.90
Threonine (g/kg)[3]	5.20	6.02	7.52	8.51	9.50
Tryptophan (g/kg)[3]	1.70	1.87	2.19	2.39	2.60
Valine (g/kg)[3]	5.20	5.77	6.82	7.51	8.20
Dispensable					
Tyrosine (g/kg)[3]	4.30	5.00	6.30	7.15	8.00

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994). [3]Concentration of amino acid on digestible basis.

Table 6. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 16-20 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
			g/kg		
Fish meal - (72%-CP)	0.00	11.31	32.13	45.82	59.50
Soybean Meal - (48%-CP)	0.00	25.3	71.9	102.6	133.2
Wheat, White	639.6	630.0	612.2	600.5	588.8
Wheat Middlings	169.60	137.38	78.02	39.01	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Casein	0.00	5.70	16.20	23.10	30.00
Soybean OiL	0.00	29.83	84.78	120.89	157.00
L-Lysine HCl	3.20	2.59	1.47	0.74	0.00
DL-Methionine	1.60	1.83	2.25	2.52	2.80
L-Threonine	0.20	0.39	0.74	0.97	1.20
Common Salt	1.40	1.34	1.24	1.17	1.10
Limestone	8.20	6.64	3.77	1.89	0.00
Dicalcium phosphate	12.50	12.54	12.61	12.65	12.70
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.48	11.52	13.43	14.69	15.95
Crude protein (g/kg)	129.5	142.5	166.5	182.3	198.0
Crude fibre (g/kg)	48.70	43.93	35.15	29.37	23.60
Ca (g/kg)	6.50	6.52	6.55	6.58	6.60
Available Phosphorus (g/kg)	3.20	3.16	3.09	3.05	3.00
Na(g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	1.90	1.81	1.63	1.52	1.40
K (g/kg)	6.20	6.09	5.88	5.74	5.60
Mn (mg/kg)	101.3	95.6	84.9	78.0	71.0
Zn (mg/kg)	100.8	95.2	84.8	78.0	71.1
Indispensable amino acids					
Arginine (g/kg)[3]	5.70	6.33	7.48	8.24	9.00
Cystine (g/kg)[3]	2.30	2.55	3.00	3.30	3.60
Isoleucine (g/kg)[3]	4.20	4.75	5.77	6.43	7.10
Lysine (g/kg)[3]	6.00	6.65	7.84	8.62	9.40
Methionine (g/kg)[3]	2.80	3.09	3.61	3.96	4.30
Phenylalanine (g/kg)[3]	4.50	5.11	6.23	6.96	7.70
Threonine (g/kg)[3]	3.50	3.90	4.63	5.12	5.60
Tryptophan (g/kg)[3]	1.50	1.63	1.88	2.04	2.20
Valine (g/kg)[3]	4.70	5.37	6.59	7.40	8.20
Dispensable					
Tyrosine (g/kg)[3]	3.80	4.39	5.47	6.19	6.90

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).[3]Concentration of amino acid on digestible basis.

Water intake

A plastic header tank with a recorded weight of water was placed on the corner of each pen for water intake determination each week for a period of 24hours. On the day of water intake determination a turkey bell drinker was attached to the header tank and after 24hours the water intake was recorded as the difference between the water offered and the water remained in the header tank at both occasions. To get the measurements of evaporative losses five bell drinker with identical volume of water were placed each day at bird height and at different points within the experimental room but out of the reach of birds. The water measurements then were recorded as kg/bird/day after correcting the evaporative losses.

Feed intake

To determine the feed intake, the feed offered at the beginning of each growth phase was recorded and the weigh back was done at the end of each phase. During the digestibility trial (on 49th day of the trial), feed intake was determined separately to get the feed intake for 24hours. The values of daily feed intake were recorded in kg/day/bird.

Body weight (BW)

Birds were weighed individually before placing them in pens to get the initial weight and then on a 4 weekly basis birds in each pen were weighed individually to get the measurements for body weight gain. This was then converted to body weight gain in kg/day/bird.

Excreta collection

For the determination of dietary nutrient digestibility coefficients (i.e. DM, CP, amino acids, minerals, organic matter, ash and metabolisable energy) excreta were collected for a period of 24hours at 7 weeks of age. Excreta were freeze-dried, weighed and milled to pass through a 0.75mm mesh.

Litter quality, Footpad and Hock score determination

A visual assessment for litter score of the entire pen was done at the end of each feeding phase (at 8, 12, 16 and 20 weeks of age). The total area of the pen was scored by attributing a percentage value to the litter which scored 1 to 5 (Da Costa *et al*., 2014). A score 1 was given to a litter that was friable, and there was no capping or compaction; score 2 was given when there was a light capping, under a friable crumb surface; when the surface was capped and compacted the score was 3; score 4 was given when the surface was wet and sticky; when the litter depth was wet and dough-like the score was 5. A percentage of each pen was allotted the appropriate score, to the nearest 5%, in the relevant score category.

Litter score were calculated and recorded as follows:

[(1 x %) + (2 x %) + (3 x %) + (4 x %) + (5 x %)]/100

A lower score will be associated with better litter quality.

Litter NH_3, temperature (T°) and pH were determined at 8, 12, 16 and 20 weeks of age by using the pH probe placed directly in to the litter and in the center of each pen (Hanna HI 99163 meter, Hanna Instruments Ltd, Bedfordshire, UK). Atmospheric ammonia was measured using a handheld Dräger meter tube (Ammonia 2/a) attached to a Dräger Multi Gas Detector pump (Draeger Safety AG and Co. KGaA, Luebeck, Germany). Ammonia concentrations were recorded from each pen, almost 3 cm above litter surface and from the central point of the pen by stroking the pump five times (approximate one minute/pen). The Dräger tubes change from yellow to blue for a positive value for ammonia.

The principle of the reaction was:

NH? + pH indicator → blue reaction product.

Litter samples were taken from the centre and mid-way between centre and four corners of each pen at the end of each growth phase. The litter samples collected were combined and homogenized in plastic bags and the moisture contents were determined by placing in an oven at 80°C for 48 hours.

Footpad and hock lesions were scored for both the left and right leg, including all birds, and classified according to a scale from Hocking *et al*. (2008) from 0 (no lesion) to 4 (very severe lesions). All birds were scored at the end of week 8, 12, 16 and 20. A composite mean of the pen was used for statistical analysis.

Amino acid determination

The amino acid content of feed and excreta was determined by High performance liquid chromatography following oxygen-free hydrochloric acid digestion (Jones *et al.*, 1981). The system comprised a Dionex ASI-100 autosampler fitted with a Dionex P580 pump and a Dionex RF-2000 detector (Sunnyrale, California, USA). The flow rate used was 1 mL min^{-1} and the column used was a Spherisorb ODS2 (150x4.6mm fitted with a Waters guard cartridge). Since this method of hydrolysis destroys methionine, cystine and tryptophan, data on these amino acids are not reported. Metabolisability coefficient for glycine is not presented because of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et al.*, 1971).

Mineral determination

The procedure followed for mineral analyses (Na, Ca, P, K, Mg, Zn and Mn) in samples of feed and excreta was the same; the digestion of samples was carried out by using Microwave Accelerated Reaction System (MARS) as used for the rapid preparation of sample for atomic absorption and the optical plasma emission spectrometry (Optima 4300 DV Dual View ICPOE spectrometer, Perkin Elmer, Beaconsfield, UK), (Tanner *et al.*, 2002).

Statistical procedure

Seven replicates per treatment were used for the experiment with a total of one hundred and seventy five turkeys. For the analysis of data, statistical measurements, average, and standard errors of differences of means were obtained for all numeric variables analysed (descriptive statistical techniques).

Randomised complete block analysis of variance (ANOVA) model, with two factors (treatment and time) for repeated measures, including the Greenhouse–Geiser degrees of freedom corrections and ANOVA for two factors, when the analysis was performed between treatments and times (inferential statistical techniques) (Zar, 1999). The model included dietary nutrient density (5 levels of dietary nutrient concentration), time (weeks ending the growth phase i.e. 8, 12, 16 and 20),and the interaction between dietary density and weeks ending the growth phases. The pens were treated as experimental units. Orthogonal polynomials were also used for average values of all numeric variables (e.g. litter moisture, litter NH_3, litter pH etc.) to compare treatment differences for linear and quadratic relationships with increasing dietary nutrient concentration. Comparison contrast test was used on the average values of all numeric variables analysed (above mentioned) to compare low nutrient density diets (i.e. 77 and 85% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation) as well as high nutrient density diets (i.e. 110 and 120% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation).

However, for data i.e. Energy efficiency ratios (EER), N excreted, N excreted as a part of amino acids and uric acid (AAN, UAN), neutral detergent fibre intake (NDF I), ash digestibility, AME and AMEn (DM basis), crude protein digestibility coefficient (CPD), dry matter digestibility coefficients (DMD) and organic matter digestibility (OMD) and amino acid intake, excretion, retention and digestibility values determined after 7^{th} weeks of birds age (at 49^{th} day of birds age). The data entered on an Excel spreadsheet and Genstat software, release 11 (IACR Rothamstead, Harpenden, Hertfordshire) was used to perform ANOVA for the comparison of different treatments for litter quality parameters i.e. moisture, NH_3, pH and temperature and other parameters such as water intake, feed intake, body weight gain, feed conversion efficiency and nutrient digestibility. Correlation coefficients were also generated on average values to test for a possible relationship between different variables. Differences were reported as significant at $P<0.05$ and trends were noted when the P value was near to 0.1.

The data obtained for FPS and HBS were compared using the values (weighted means for each pen for TFPS and THS) for each pen for good hock (GHS), bad hock (BHS), total hock (THS) scores and for good footpad (GFPS), bad footpad (BFPS) and total footpad (TFPS) scores, by using ANOVA for the comparison of different treatments. There were not enough different non zero scores to make a multinomial analyses (or chi-squared) possible for FPS and HBS data (real values) and also, it was not possible to incorporate the random structure in the data using Chi-squared, however, since the residual plot were unacceptable after running Residual maximum likelihood (REML). Therefore, generalized linear mixed models (GLMM), were fitted using residual maximum likelihood (REML) to binary data: FPD>0, or not, and HB>0, or not (binomial, link logit transformed) and fixed effects time+treatment and random effects bird weight category, block and pen with dispersion fixed at 1. There was not enough information in the data to include the interaction term (i.e. time x treatment). The P-values, estimated means, SEMs and back transformed means are reported in the result tables. Since no FP lesions appeared at the end of week 8 the data for FPS, this time point was not included in analysis.

RESULTS

The birds remained healthy and overall mortality was less than 1% throughout the experiment, with no significant difference between treatment groups (data not shown).

The Analysed chemical composition of the basal diets is presented in tables (table 7 to 10). The analysed values for the concentration of CP content were lower than the calculated values in table 3 to 6, however, the analysed values for K, Ca and Na concentration were higher than the calculated values. Digestible amino acid data taken from the literature was derived from studies on the birds of varying breed, sex and age as well as method of digestibility determination (ileal and total tract). In contrast the data collected during the course of this study has been obtained from controlled groups of birds of same breed, sex and age as well as using total tract method for digestibility determination, so no comparison is made here.

Water intake measurements

Increased nutrient density had a negative effect on water intake (WI) and feed intake used for water:feed determination (feed intake measured for 24 hours time period to determine water:feed, FI W:F) which decreased linearly ($P<0.01$ and 0.001, respectively) as the density increased (table 16). However there was no effect ($P>0.05$) of the dietary nutrient density recorded on water:feed (W:F). The WI, FI W:F linearly increased ($P<0.001$) with the increase of the age of the birds, the WI and FI W:F values were observed during the last feeding phase of the study. The increase of the birds age had a negative effect ($P<0.01$) on W:F and the lowest values were recorded in the last two feeding phases of the study (table 16). The results for WI, FI W:F and W:F were subject to a dietary density x time interaction ($P<0.001$ for WI and $P<0.05$ for the rest), showing that the responses to feed density were different during growing periods. For example, an increase in nutrient density during the first feeding phase led to an increase in WI, although the response during the rest of the feeding phases was the opposite and the WI decreased when nutrient density increased. An increase in dietary density did not have significant effect on the FI W:F during the first two feeding phases, but led to a decrease FI during the last two feeding phases. Dietary density increased W:F during the first feeding phase, although the responses of W:F were inconsistent for the rest of the study.

Table 7. Analysed composition of experimental diets for 4-8 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	868.8	868.9	869.2	869.3	869.5
Crude protein (g/kg)	193.2	215.7	257.2	284.4	312.1
Gross energy (MJ/kg)	16.27	16.77	17.70	18.31	18.94
Ash (g/kg)	64.74	64.92	65.26	65.48	65.77
Crude fat (g/kg)	30.24	46.95	77.73	97.96	118.32
Neutral detergent fibre (g/kg)	99.94	89.10	69.15	56.04	42.98
Ca (g/kg)	11.64	11.36	10.85	10.51	10.18
Total Phosphorous (g/kg)	8.64	8.68	8.76	8.81	8.87
Na (g/kg)	1.13	1.26	1.51	1.67	1.83
K (g/kg)	9.56	9.89	10.50	10.90	11.31
Cu (mg/kg)	19.55	19.68	19.93	20.09	20.27
Mg (g/kg)	2.00	1.97	1.90	1.86	1.83
Mn (mg/kg)	139.0	135.2	128.3	123.7	119.2
Zn (mg/kg)	125.1	128.3	134.1	137.9	141.8
Indispensable amino acids					
Arginine (g/kg)	9.84	11.01	13.16	14.57	16.01
Histidine (g/kg)	3.56	4.03	4.90	5.48	6.06
Isoleucine (g/kg)	8.32	9.49	11.63	13.04	14.47
Leucine (g/kg)	13.59	15.43	18.83	21.06	23.32
Lysine (g/kg)	10.62	12.06	14.71	16.45	18.21
Methionine (g/kg)	3.14	3.59	4.41	4.96	5.51
Phenylalanine (g/kg)	8.98	10.04	11.99	13.27	14.56
Threonine (g/kg)	7.02	8.19	10.34	11.75	13.18
Valine (g/kg)	8.80	9.93	12.01	13.37	14.76
Dispensable					
Alanine (g/kg)	6.95	7.93	9.73	10.91	12.11
Aspartic acid (g/kg)	16.85	19.20	23.52	26.36	29.23
Glutamic acid (g/kg)	39.98	43.55	50.13	54.46	58.85
Glycine (g/kg)	5.96	6.84	8.47	9.55	10.63
Serine (g/kg)	6.01	6.88	8.49	9.55	10.62
Tyrosine (g/kg)	5.01	5.72	7.03	7.89	8.76

Table 8. Analysed composition of experimental diets for 8-12 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	850.9	849.7	847.3	845.8	844.3
Crude protein (g/kg)	156.3	176.8	214.1	238.7	263.0
Gross energy (MJ/kg)	15.87	16.51	17.67	18.44	19.19
Ash (g/kg)	59.57	59.08	58.10	57.53	56.89
Crude fat (g/kg)	23.83	45.60	85.46	111.63	137.57
Ca (g/kg)	9.62	9.49	9.25	9.10	8.95
Total Phosphorous (g/kg)	7.98	7.88	7.68	7.56	7.44
Na (g/kg)	0.60	0.74	1.00	1.18	1.35
K (g/kg)	7.74	7.99	8.44	8.74	9.03
Cu (mg/kg)	16.08	16.50	17.24	17.75	18.23
Mg (g/kg)	1.96	1.91	1.81	1.75	1.69
Mn (mg/kg)	120.8	118.8	114.8	112.3	109.7
Zn (mg/kg)	124.3	128.5	136.0	141.1	146.0
Indispensable amino acids					
Arginine (g/kg)	6.73	7.93	10.11	11.55	12.97
Histidine (g/kg)	2.57	3.08	4.02	4.64	5.25
Isoleucine (g/kg)	5.96	7.18	9.41	10.89	12.34
Leucine (g/kg)	10.31	12.34	16.03	18.47	20.87
Lysine (g/kg)	8.60	9.78	11.92	13.33	14.73
Methionine (g/kg)	3.11	3.59	4.46	5.04	5.60
Phenylalanine (g/kg)	6.60	7.84	10.10	11.59	13.07
Threonine (g/kg)	4.77	5.94	8.06	9.46	10.85
Valine (g/kg)	6.83	7.89	9.82	11.09	12.35
Dispensable					
Alanine (g/kg)	5.17	6.06	7.68	8.75	9.80
Aspartic acid (g/kg)	11.52	14.08	18.76	21.84	24.89
Glutamic acid (g/kg)	30.74	34.65	41.77	46.47	51.10
Glycine (g/kg)	5.12	6.05	7.75	8.86	9.97
Serine (g/kg)	4.37	5.21	6.74	7.75	8.75
Tyrosine (g/kg)	3.53	4.26	5.58	6.45	7.31

Table 9. Analysed composition of experimental diets for 12-16 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	849.3	849.8	850.6	851.2	851.7
Crude protein (g/kg)	138.1	156.8	191.1	213.6	236.3
Gross energy (MJ/kg)	15.75	16.38	17.51	18.25	19.01
Ash (g/kg)	51.45	51.87	52.58	53.01	53.51
Crude fat (g/kg)	20.12	40.87	79.13	104.2	129.5
Ca (g/kg)	8.66	8.75	8.91	9.01	9.12
Total Phosphorous (g/kg)	7.37	7.39	7.43	7.45	7.48
Na (g/kg)	0.68	0.76	0.91	1.01	1.11
K (g/kg)	6.79	6.93	7.18	7.33	7.50
Cu (mg/kg)	18.08	19.49	22.08	23.76	25.47
Mg (g/kg)	1.70	1.64	1.52	1.44	1.36
Mn (mg/kg)	124.8	126.6	129.7	131.7	133.8
Zn (mg/kg)	114.6	116.7	120.4	122.8	125.2
Indispensable amino acids					
Arginine (g/kg)	5.90	6.92	8.79	10.01	11.25
Histidine (g/kg)	2.42	2.85	3.64	4.16	4.69
Isoleucine (g/kg)	5.31	6.28	8.05	9.21	10.38
Leucine (g/kg)	9.20	10.66	13.35	15.10	16.88
Lysine (g/kg)	8.57	9.68	11.73	13.08	14.43
Methionine (g/kg)	3.89	4.44	5.44	6.10	6.76
Phenylalanine (g/kg)	6.16	7.01	8.58	9.61	10.65
Threonine (g/kg)	4.56	5.58	7.47	8.70	9.95
Valine (g/kg)	6.65	7.62	9.41	10.58	11.77
Dispensable					
Alanine (g/kg)	4.71	5.53	7.04	8.03	9.03
Aspartic acid (g/kg)	9.64	11.62	15.27	17.66	20.07
Glutamic acid (g/kg)	32.21	35.43	41.34	45.20	49.12
Glycine (g/kg)	4.80	5.72	7.41	8.52	9.64
Serine (g/kg)	3.98	4.73	6.10	7.00	7.91
Tyrosine (g/kg)	2.90	3.41	4.36	4.99	5.61

Table 10. Analysed composition of experimental diets for 16-20 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	849.7	851.3	854.2	856.2	858.1
Crude protein (g/kg)	120.0	133.7	159.3	176.1	193.1
Gross energy (MJ/kg)	15.77	16.42	17.64	18.45	19.27
Ash (g/kg)	46.41	45.85	44.88	44.23	43.59
Crude fat (g/kg)	20.06	44.73	90.44	120.65	151.01
Ca (g/kg)	8.50	8.40	8.22	8.10	7.98
Total Phosphorous (g/kg)	6.72	6.79	6.91	7.00	7.08
Na (g/kg)	0.77	0.83	0.95	1.03	1.12
K (g/kg)	6.04	6.04	6.06	6.08	6.09
Cu (mg/kg)	17.68	17.28	16.56	16.09	15.62
Mg (g/kg)	1.62	1.54	1.39	1.30	1.20
Mn (mg/kg)	123.3	121.9	119.7	118.2	116.7
Zn (mg/kg)	122.4	124.8	129.4	132.5	135.6
Indispensable amino acids					
Arginine (g/kg)	4.65	5.32	6.58	7.41	8.25
Histidine (g/kg)	2.04	2.27	2.70	2.99	3.28
Isoleucine (g/kg)	4.30	5.10	6.59	7.57	8.55
Leucine (g/kg)	7.76	8.95	11.15	12.61	14.07
Lysine (g/kg)	5.96	6.59	7.77	8.55	9.34
Methionine (g/kg)	1.92	2.40	3.29	3.88	4.47
Phenylalanine (g/kg)	5.29	5.98	7.26	8.11	8.97
Threonine (g/kg)	2.55	3.12	4.19	4.89	5.60
Valine (g/kg)	5.12	5.91	7.38	8.35	9.33
Dispensable					
Alanine (g/kg)	3.74	4.30	5.33	6.01	6.70
Aspartic acid (g/kg)	7.34	8.92	11.87	13.81	15.77
Glutamic acid (g/kg)	29.39	31.68	35.94	38.76	41.60
Glycine (g/kg)	4.15	4.89	6.27	7.18	8.09
Serine (g/kg)	3.21	3.66	4.51	5.06	5.62
Tyrosine (g/kg)	2.08	2.50	3.26	3.77	4.28

Table 11. Effect of dietary nutrient concentration and time on litter moisture (LM), litter ammonia (NH_3, ppm), litter pH (pH), litter temperature (T°) and litter score (LS) parameters.

	Treatments		LM	NH_3	pH	T°	LS
	Diets						
	T1		362.5	6.57	7.74	20.74	2.08
	T2		328.9	6.81	7.85	20.45	1.88
	T3		328.2	8.53	8.21	20.37	1.75
	T4		297.8	8.87	8.15	20.61	1.70
	T5		280.5	9.50	8.12	20.69	1.59
SEM			29.05	0.371	0.069	0.119	0.129
	Time (wks)						
	4-8		225.6	3.21	7.63	21.02	1.43
	8-12		318.0	14.42	8.58	19.83	1.80
	12-16		358.5	9.69	8.13	20.52	2.03
	16-20		376.2	4.90	7.71	20.92	1.94
SEM			9.52	0.268	0.070	0.121	0.044
	Diets	**Time (wks)**					
	T1	4-8	244.0	2.91	7.69	20.98	1.50
	T2	4-8	236.2	3.16	7.49	21.21	1.47
	T3	4-8	232.1	3.73	8.01	20.80	1.44
	T4	4-8	208.7	2.63	7.49	21.11	1.40
	T5	4-8	207.1	3.59	7.47	21.00	1.36
	T1	8-12	348.4	12.50	8.37	20.26	2.07
	T2	8-12	335.1	13.14	8.42	19.61	2.06
	T3	8-12	318.0	14.84	8.64	19.69	1.70
	T4	8-12	302.5	15.07	8.76	19.51	1.69
	T5	8-12	286.0	16.54	8.71	20.06	1.49
	T1	12-16	422.2	7.07	7.53	20.66	2.27
	T2	12-16	355.4	7.07	7.94	20.31	2.15
	T3	12-16	377.8	10.81	8.39	20.19	2.11
	T4	12-16	323.3	10.79	8.40	20.74	1.85
	T5	12-16	313.6	12.71	8.40	20.69	1.76
	T1	16-20	435.5	3.79	7.37	21.06	2.49
	T2	16-20	388.7	3.86	7.55	20.64	1.83
	T3	16-20	384.8	4.71	7.79	20.79	1.76
	T4	16-20	356.7	7.00	7.97	21.09	1.84
	T5	16-20	315.4	5.14	7.88	21.03	1.75
SEM			27.60	0.638	0.152	0.263	0.129
	Probabilities of statistical differences						
Diets			P=0.08	<0.001	<0.001	NS	<0.05
Linear			<0.01	<0.001	NS	NS	<0.001
Quadratic			NS	NS	P=0.06	NS	NS
Contrast 1			NS	<0.001	NS	NS	P=0.07
Contrast 2			NS	NS	NS	NS	NS
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			NS	<0.01	NS	NS	<0.05

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 12. Effect of dietary nutrient concentration and time on leg health parameters i.e. good hock score (GHS), bad hock score (BHS) and total hock score (THS).

Treatments		GHS	BHS	THS
Diets				
T1		0.721	0.279	0.329
T2		0.829	0.171	0.302
T3		0.657	0.343	0.491
T4		0.670	0.330	0.462
T5		0.559	0.441	0.868
SEM		0.0607	0.0607	0.1150
Time (wks)				
4-8		0.456	0.544	0.726
8-12		0.696	0.304	0.501
12-16		0.811	0.189	0.333
16-20		0.559	0.214	0.401
SEM		0.0324	0.0324	0.0493
Diets	**Time (wks)**			
T1	4-8	0.543	0.457	0.543
T2	4-8	0.600	0.400	0.571
T3	4-8	0.500	0.500	0.621
T4	4-8	0.314	0.686	0.800
T5	4-8	0.321	0.679	1.093
T1	8-12	0.757	0.243	0.300
T2	8-12	0.807	0.193	0.371
T3	8-12	0.664	0.336	0.486
T4	8-12	0.771	0.229	0.286
T5	8-12	0.479	0.521	1.064
T1	12-16	0.779	0.221	0.250
T2	12-16	0.936	0.064	0.150
T3	12-16	0.814	0.186	0.314
T4	12-16	0.800	0.200	0.371
T5	12-16	0.729	0.271	0.579
T1	16-20	0.807	0.193	0.221
T2	16-20	0.971	0.029	0.114
T3	16-20	0.650	0.350	0.543
T4	16-20	0.793	0.207	0.393
T5	16-20	0.707	0.293	0.736
SEM		0.0873	0.0873	0.1495
Probabilities of statistical differences				
Diets		P=0.06	P=0.06	<0.05
Linear		<0.05	<0.05	<0.01
Quadratic		Ns	NS	NS
Contrast 1		NS	NS	NS
Contrast 2		NS	NS	NS
Time		<0.001	<0.001	<0.001
Diets x Time		NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 13. Effect of dietary nutrient concentration and time on leg health parameters i.e. good footpad score (GFPS), bad footpad score (BFPS) and total footpad score (TFPS).

Treatments		GFPS	BFPS	TFPS
Diets				
T1		0.876	0.124	0.167
T2		0.879	0.121	0.160
T3		0.867	0.133	0.117
T4		0.857	0.143	0.226
T5		0.905	0.095	0.105
SEM		0.0471	0.0471	0.0805
Time (wks)				
4-8		--	--	--
8-12		0.721	0.279	0.350
12-16		0.970	0.030	0.036
16-20		0.939	0.061	0.079
SEM		0.0308	0.0308	0.0405
Diets	**Time (wks)**			
T1	4-8	--	--	--
T2	4-8	--	--	--
T3	4-8	--	--	--
T4	4-8	--	--	--
T5	4-8	--	--	--
T1	8-12	0.750	0.250	0.350
T2	8-12	0.729	0.271	0.357
T3	8-12	0.664	0.336	0.286
T4	8-12	0.714	0.286	0.479
T5	8-12	0.750	0.250	0.279
T1	12-16	1.000	0.000	0.000
T2	12-16	0.971	0.029	0.029
T3	12-16	0.971	0.029	0.029
T4	12-16	0.943	0.057	0.086
T5	12-16	0.964	0.036	0.036
T1	16-20	0.879	0.121	0.150
T2	16-20	0.936	0.064	0.093
T3	16-20	0.964	0.036	0.036
T4	16-20	0.914	0.086	0.114
T5	16-20	1.000	0.000	0.000
SEM		0.0734	0.0734	0.1090
Probabilities of statistical differences				
Diets		NS	NS	NS
Linear		NS	NS	NS
Quadratic		NS	NS	NS
Contrast 1		NS	NS	NS
Contrast 2		NS	NS	NS
Time		<0.001	<0.001	<0.001
Diets x Time		NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 14. Effect of dietary nutrient concentration and time on leg health parameters i.e. incidences of hock burn (HB) and incidences of footpad dermatitis (FPD), from generalized linear mixed models (GLMM) on logit scale and back transformed on proportion scale (i.e. % of birds with HB>0, FPD>0).

	Treatments	Logit of HB Incidence	Incidence of HB>0	Logit of FPD Incidence	Incidence of FPD>0
	Diets				
	T1	-1.317	21.13	-2.632	6.71
	T2	-2.057	11.33	-2.527	7.40
	T3	-0.799	31.03	-2.856	5.44
	T4	-0.970	27.49	-2.408	8.25
	T5	-0.308	42.37	-2.828	5.58
Min and max SEM		0.5121-0.5510		0.5528-0.5915	
	Time (wks)				
	4-8	0.225	55.59	--	--
	8-12	-1.104	24.89	-1.200	23.15
	12-16	-1.830	13.83	-3.758	2.28
	16-20	-1.651	16.10	-2.993	4.77
Min and max SEM		0.4231-0.4458		0.2772-0.5117	
	Probabilities of statistical differences				
Diets		<0.05		NS	
Time		<0.001		<0.001	

There is a statistical significant difference when P<0.05; SEM- standard errors of means (min= Minimum and max= Maximum). The p-values and SEMs are associated with the estimated means on the logit scale of the analysis.

Table 15. Effect of dietary nutrient concentration, time (growth phases) and their interaction on total weight gain ((TWG) kg/b/4 weeks), weight gain ((WG) kg/b/d), feed intake ((FI) kg/b/d), feed conversion efficiency ((FCE) wt gain kg/kg FI) and protein efficiency ratio (PER, wt gain kg/CP intake g).

Treatments		TWG	WG	FI	FCE	PER
Diets						
T1		4.12	0.147	0.479	0.354	1.84
T2		4.45	0.159	0.519	0.359	1.96
T3		4.57	0.163	0.462	0.401	2.03
T4		4.49	0.160	0.433	0.417	2.13
T5		4.66	0.166	0.410	0.453	2.12
SEM		0.078	0.0028	0.0146	0.0072	0.105
Time (wks)						
4-8		3.34	0.119	0.201	0.597	2.49
8-12		5.00	0.179	0.429	0.419	2.14
12-16		5.15	0.184	0.600	0.311	1.78
16-20		4.34	0.155	0.613	0.259	1.66
SEM		0.051	0.0018	0.0069	0.0045	0.033
Diets	**Time (wks)**					
T1	4-8	3.18	0.114	0.208	0.551	2.34
T2	4-8	3.25	0.116	0.211	0.554	2.42
T3	4-8	3.32	0.119	0.201	0.592	2.40
T4	4-8	3.41	0.122	0.194	0.629	2.62
T5	4-8	3.53	0.126	0.192	0.659	2.68
T1	8-12	4.62	0.165	0.446	0.372	1.96
T2	8-12	4.92	0.176	0.456	0.387	2.05
T3	8-12	5.09	0.182	0.425	0.428	2.08
T4	8-12	5.10	0.182	0.420	0.434	2.30
T5	8-12	5.26	0.188	0.396	0.477	2.29
T1	12-16	5.02	0.179	0.632	0.287	1.65
T2	12-16	5.12	0.183	0.663	0.277	1.69
T3	12-16	5.09	0.182	0.583	0.314	1.87
T4	12-16	5.20	0.186	0.582	0.321	1.87
T5	12-16	5.30	0.189	0.541	0.356	1.81
T1	16-20	3.65	0.130	0.632	0.207	1.42
T2	16-20	4.52	0.161	0.747	0.217	1.66
T3	16-20	4.75	0.170	0.640	0.268	1.78
T4	16-20	4.24	0.152	0.534	0.285	1.73
T5	16-20	4.55	0.163	0.512	0.319	1.71
SEM		0.126	0.0045	0.0198	0.0113	0.123
Probabilities of statistical differences						
Diets		<0.001	<0.001	<0.001	<0.001	NS
Linear		<0.001	<0.001	<0.001	<0.001	<0.05
Quadratic		NS	NS	NS	NS	NS
Contrast 1		<0.01	<0.01	<0.05	<0.001	NS
Contrast 2		NS	NS	<0.05	<0.001	NS
Time		<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time		<0.01	<0.01	<0.001	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 16. Effect of dietary nutrient concentration, time (growth phases) and their interaction on water intake ((WI) kg/b/d), feed intake for water ratio feed (FI W:F) kg/b/d) and water ratio feed ((W:F) kg/kg).

Treatments		WI	FI W:F	W:F
Diets				
T1		0.843	0.500	1.73
T2		0.823	0.518	1.69
T3		0.791	0.479	1.75
T4		0.738	0.458	1.72
T5		0.684	0.402	1.81
SEM		0.0381	0.0191	0.050
Time (wks)				
4-8		0.471	0.219	2.15
8-12		0.788	0.449	1.76
12-16		0.855	0.581	1.48
16-20		0.989	0.635	1.57
SEM		0.0180	0.0101	0.029
Diets	**Time (wks)**			
T1	4-8	0.439	0.227	1.93
T2	4-8	0.459	0.222	2.07
T3	4-8	0.452	0.209	2.15
T4	4-8	0.501	0.224	2.24
T5	4-8	0.506	0.214	2.36
T1	8-12	0.792	0.471	1.69
T2	8-12	0.841	0.478	1.77
T3	8-12	0.858	0.459	1.86
T4	8-12	0.736	0.432	1.71
T5	8-12	0.711	0.402	1.77
T1	12-16	1.004	0.640	1.58
T2	12-16	0.922	0.629	1.48
T3	12-16	0.832	0.581	1.44
T4	12-16	0.767	0.551	1.40
T5	12-16	0.752	0.505	1.50
T1	16-20	1.136	0.660	1.73
T2	16-20	1.070	0.742	1.45
T3	16-20	1.023	0.665	1.53
T4	16-20	0.946	0.624	1.52
T5	16-20	0.768	0.486	1.61
SEM		0.0516	0.0279	0.075
Probabilities of statistical differences				
Diets		<0.05	<0.01	NS
Linear		<0.01	<0.001	NS
Quadratic		NS	P=0.09	NS
Contrast 1		NS	NS	NS
Contrast 2		NS	<0.05	NS
Time		<0.001	<0.001	<0.001
Diets x Time		<0.001	<0.01	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 17. The effect of dietary protein and energy on growth performance, water intake, litter quality and nutrient utilisation parameters of turkeys

	Dietary treatments					Probabilities of significant differences					
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**	**SEM**	**P**	**Linear**	**Quadratic**	**Contrast 1**	**Contrast 2**
Energy efficiency ratio (EER, kg/MJ)	0.054	0.036	0.032	0.034	0.028	0.0056	<0.05	<0.01	NS	P=0.06	NS
N Excreted (g/b/d)	3.810	3.867	4.775	5.184	5.945	0.3170	<0.001	<0.001	NS	<0.05	P=0.05
AAN (g/b/d)	0.935	1.406	1.586	1.599	2.170	0.1586	<0.001	<0.001	NS	<0.05	NS
UAN (g/b/d)	1.521	2.461	3.189	3.585	3.775	0.1934	<0.001	<0.001	<0.05	<0.001	<0.05
NDF I (g/b/d)	18.03	16.29	12.08	9.47	7.17	0.366	<0.001	<0.001	NS	<0.001	<0.001
AME (MJ/kg)	11.53	13.43	15.17	16.04	17.44	0.422	<0.001	<0.001	NS	<0.001	<0.01
AMEn (MJ/kg)	10.92	12.62	14.20	15.04	16.24	0.542	<0.001	<0.001	NS	<0.001	<0.01
AME I (MJ/b/d)	2.07	2.46	2.65	2.71	2.91	0.084	<0.001	<0.001	NS	<0.001	NS
CPD	0.499	0.595	0.597	0.554	0.609	0.0293	P=0.081	P=0.08	NS	NS	NS
DMD	0.587	0.664	0.701	0.709	0.746	0.0241	<0.001	<0.001	NS	<0.05	NS
OMD	0.622	0.690	0.724	0.731	0.766	0.0221	<0.001	<0.001	NS	<0.05	NS

Energy efficiency ratios (EER), N excreted, N excreted as a part of amino acids and uric acid (AAN, UAN), ash digestibility, AME and AMEn (DM basis), crude protein digestibility coefficient (CPD), dry matter digestibility coefficients (DMD) and organic matter digestibility (OMD) were determined at 49th days of age. However, AME I values represents for growth phase 4-8 weeks were obtained on dry matter basis. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 18. The effect of dietary protein and energy on total tract amino acid digestibility coefficients by turkeys at 8 weeks of age.

	Dietary treatments					Probabilities of significant differences					
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**	**SEM**	**P**	**Linear**	**Quadratic**	**Contrast 1**	**Contrast 2**
Alanine	0.730	0.782	0.821	0.843	0.871	0.0133	<0.001	<0.001	NS	<0.001	<0.05
Arginine	0.856	0.873	0.903	0.910	0.921	0.0080	<0.001	<0.001	NS	<0.001	NS
Aspartic acid	0.766	0.818	0.842	0.866	0.872	0.0164	<0.001	<0.001	NS	<0.05	NS
Glutamic acid	0.864	0.888	0.895	0.895	0.911	0.0083	<0.01	<0.001	NS	P=0.06	NS
Histidine	0.838	0.867	0.887	0.900	0.894	0.0136	<0.05	<0.01	NS	<0.05	NS
Isoleucine	0.782	0.825	0.856	0.859	0.883	0.0135	<0.001	<0.001	NS	<0.01	NS
Leucine	0.781	0.827	0.858	0.859	0.905	0.0147	<0.001	<0.001	NS	<0.01	NS
Lysine	0.834	0.864	0.896	0.900	0.917	0.0093	<0.001	<0.001	NS	<0.001	NS
Phenylalanine	0.783	0.826	0.852	0.840	0.870	0.0118	<0.001	<0.001	NS	<0.01	NS
Serine	0.819	0.849	0.877	0.879	0.895	0.0102	<0.001	<0.001	NS	<0.01	NS
Threonine	0.805	0.845	0.871	0.874	0.892	0.0099	<0.001	<0.001	NS	<0.001	NS
Tyrosine	0.816	0.857	0.881	0.889	0.905	0.0104	<0.001	<0.001	NS	<0.01	NS
Valine	0.731	0.787	0.822	0.831	0.868	0.0163	<0.001	<0.001	NS	<0.01	NS

Amino acids digestibilities were determined at 49th days of age. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 19. Correlation matrix for bird performance, litter quality, dietary nutrient digestibility, and leg health in response changes in nutrient density**.**

	FI	WG	FCE	WI	W:F	LS	LM	NH_3	CPD	DMD	HBS
WG	-0.490										
FCE	-0.918	0.787									
WI	0.890	-0.757	-0.980								
W:F	-0.808	0.486	0.796	-0.733							
LS	0.732	-0.941	-0.933	0.920	-0.595						
LM	0.737	-0.846	-0.915	0.959	-0.549	0.955					
NH_3	-0.882	0.817	0.972	-0.935	0.671	-0.953	-0.900				
CPD	-0.176	0.929	0.545	-0.522	0.344	-0.760	-0.657	0.552			
DMD	-0.666	0.968	0.899	-0.885	0.555	-0.996	-0.940	0.924	0.814		
HBS	-0.831	0.709	0.922	-0.906	0.930	-0.810	-0.806	0.813	0.561	0.781	
FPS	0.128	-0.415	-0.283	0.185	-0.663	0.252	0.106	-0.167	-0.560	-0.280	-0.557

d.f. = 33 Correlation coefficients greater than 0.349 and 0.449 are statistically significant at 5% ($P<0.05$) and 1% level ($P<0.001$), respectively.
Key:FI (feed intake), WG (weight gain), FCE (feed conversion efficiency), WI (water intake), W:F (water to feed ratio), LS (litter score), LM (litter moisture content), NH_3 (ammonia in litter), CPD (crude protein digestibility), DMD (dry matter digestibility), HBS (hock burn scores) and FPS (footpad dermatitis scores).

Litter quality associated parameters

Increased nutrient density had a negative effect on litter moisture (LM), and litter score (LS) which decreased in a linear way (P<0.01 and 0.001, respectively) as the density increased (table 11). However, the LM and LS linearly increased (P<0.001) with the increase of the age of the birds, the highest LM and LS values were observed during the last feeding phases of the study. Increased nutrient density had a positive effect on litter ammonia (NH_3) which increased in a linear way (P<0.001) as the density increased (table 11). The time response of litter NH_3 concentration was also quadratic (P<0.01) as the highest values were observed for the second (8-12 week) and third (12-16 week) growing phases. Litter pH tended (P=0.06) to have a quadratic response to dietary density. The time response of litter pH was also quadratic (P<0.001) as the highest values were observed for the second (8-12 week) and third (12-16 week) growing phases. Litter temperature (T°) was not affected by dietary density (P>0.05) but responded in a quadratic manner to time as the lowest T° was observed between 8-12 weeks of age. The results for litter ammonia and litter score (NH_3 and LS, respectively) were subject to a dietary density x time interaction (P<0.05), showing that there were different patterns of response during different growing phases. For example, the response of the LS to diets T4 and T5 seems not to be influenced by the feeding phase although the response of feeding the rest of the diets tended to follow a quadratic pattern. The response of litter NH_3 to dietary density during different feeding phases was also inconsistent. The comparison contrast test did not find a difference in LM, pH, T° and LS between diet T3 and low nutrient density group (T1 and T2) as well as diet T3 and higher nutrient density group (T4 and T5). However, significantly higher litter $NH_3$was recorded in groups fed the control diet when compared with groups fed lower nutrient density diets, whereas, no difference (P>0.05) was recorded when the control diet fed group was compared with higher nutrient density fed groups.

Leg health parameters

As nutrient density increased so did the prevalence of hock burn (P<0.05). Increasing nutrient density had a negative linear effect (P<0.05) on good hock scores (GHS). It, however, resulted in a linear increase in bad hock scores (BHS) and total hock scores (THS) (P<0.05 and P<0.01, respectively) (table 12). The growth phases had significant effect (P<0.001) on all hock score parameters, where GHS increased with growth phases, conversely BHS and THS decreased as the bird aged. There was no time and diets interaction noted (P>0.05) for hock burn parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05). There was no effect of nutrient densities observed (P>0.05) for the footpad quality score (table 13). However, growth phase had a significant effect (P<0.001) on all foot score parameters, where good footpad scores (GFPS) increased with growth phases, conversely bad footpad scores (BFPS) and total footpad scores (TFPS) decreased (P<0.001) as the birds aged. There was no time by diets interaction noted (P>0.05) for footpad quality parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05) (table 13).

As for hock burn (HB) the results obtained showed an increase in HB incidence in birds fed diet containing higher nutrient density (P<0.05). However, there was a significant decrease (P<0.001) in the incidence of HB as birds grew older 56% vs. 16% birds with HB>0 at the end of week 8 and 20, respectively. The incidence of footpad dermatitis (FPD) however, was not affected by treatment (P>0.05). However, the effect of time period was significant (P<0.001) for both HB and FPD as there were higher incidences recorded at the end of weeks 8 and 12, respectively which fell at the end of week 16 with an increase at week 20.

Correlations between variables are shown in (table 19). Hock burn score (HBS) was associated with many of the parameters and in particular water to feed ratio (r = 0.930; P<0.001), feed conversion efficiency (r = 0.922; P<0.001), water intake (r = -0.906; P<0.001) and ammonia in litter (r = 0.813; P<0.001). Interestingly, footpad score (FPS) was only associated with the water to feed ratio (r = - 0.663; P<0.001).

Growth performance, dietary nutrient intake and utilisation

Overall body weight (BW) was higher than the breed standards at 20 weeks of age, i.e. 18.81 kg vs. target of 15.18 kg (data not included in tables). Increased nutrient density had a positive effect on total weight gain (TWG), weight gain (WG) and feed conversion efficiency (FCE) which increased following a linear pattern (P<0.001) when density increased (table 15). Increasing nutrient density had a negative linear effect (P<0.001) on feed intake (FI). TWG and WG increase (P<0.001) with the increase in the age of the birds whereas FCE decreased linearly (P<0.001) with the increase in the age of the birds. The protein efficiency ratio (PER) response to feed density was also linear (P<0.05) and as expected, the PER decreased (P<0.001) with age. The FCE value for the control diet was higher (P<0.001) than the lower nutrient density fed group, and lower (P<0.001) than the higher nutrient density fed group, respectively (table 15). The results for TWG, WG and FI were subject to a dietary density x time interaction (P<0.001), showing that the responses to feed density differed with age. The response of TWG and WG to nutrient density was linear (P<0.001) during the growth phases consist of 4-8 and 8-12 weeks. While a non-significant (P>0.05) effect of dietary nutrient density on these parameters were recorded during 12-16 weeks time period, whereas, the response of these parameters to dietary nutrient density was quadratic (P<0.05) during time period 16-20 weeks. The response of FI to nutrient density was linear (P<0.001) during growth phases consisting of 4-8, 8-12 and 12-16 weeks. Whereas, the response of FI to dietary nutrient density was quadratic (P<0.05) from 16-20 weeks.

Nutrient density had a positive and linear effect (P<0.001) on dry matter digestibility (DMD) and

organic matter digestibility (OMD), whereas the effect of nutrient density on dietary crude protein digestibility (CPD) only approached significance (P=0.081) (table 17). No difference (P>0.05) existed for the CPD when the comparison was made between birds fed control diet (T3-100% of standard breed recommendation) and lower nutrient density (T1and T2, 77 and 85% of standard breed recommendation, respectively), and control diet fed vs. higher nutrient density diets (T4 and T5, 110 and 120% of standard breed recommendation, respectively) fed birds. Control diet fed birds had higher (P<0.01) DMD and OMD almost 12 and 10%, in comparison to birds offered the lower nutrient concentration diets. However, no difference (P>0.05) in DMD and OMD amongst birds existed when the comparison was made between the control diet and higher nutrient density diets.

Increasing dietary nutrient concentration led to a linear (P<0.001) improvement in apparent metabolisable energy (AME) and apparent metabolisable energy corrected to nitrogen (AMEn) values of the diets, as AME and AMEn values were reduced for diets T1, T2, T3 and T4 ranged from 34 to 8% lower as compared to T5 diet. Birds fed control diet had higher (P<0.001) dietary AME and AMEn values in comparison to birds offered the lower nutrient concentration diets. However, AME and AMEn values were 9% lower (P<0.01) for the control diet, compared with higher nutrient density fed birds (table 17). The response of AME intake (AME I) to dietary nutrient concentration was a linear function (P<0.01), where AME I increased with higher dietary nutrient concentration. Birds fed control diet had higher (P<0.001) AME I values in comparison to birds offered the lower nutrient concentration diets, however, no difference (P>0.05) in AME I amongst birds existed when the comparison was made between the control diet and higher nutrient density diets (table 17).

There was a linear increase (P<0.001) in nitrogen excretion (NEx), nitrogen excretion as part of amino acids (AAN) and nitrogen excretion as uric acid (UAN) as nutrient density increased. On the contrary energy efficiency ratio (EER) positively increased (P<0.001) with lower dietary nutrient concentration, similarly intake of neutral detergent fibre (NDF) increased with a decrease in dietary nutrient density (table 17). Birds fed diet T1 had significantly higher intake of NDF (P<0.001), almost 134% higher, when compared with the birds fed diet T5. There was a significantly higher (P<0.05) NEx, AAN and UAN was noted when control diet fed birds were compared with lower and higher nutrient density diets fed birds, however, the difference was not significant (P>0.05) for the AAN when comparisons were made between control diet and higher nutrient density diets fed birds. There was no difference in EER between the control diet and lower and higher nutrient density diets fed birds. The intake of NDF was significantly higher (P<0.05) when comparisons were made between the control diet and lower nutrient density diets, however, there was a significantly (P<0.001) lower intake of NDF when the control diet was compared with high nutrient density diet.

Overall the response of amino acid digestibility (during digestibility measurements after 7th week at 49 days of birds age) i.e. for Ala, Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Ser, Thr, Tyr and Val was best described as positive linear function (P<0.001) to dietary nutrient concentration (table 18). Birds fed the control diet had higher (P<0.001) amino acid digestibility in comparison to birds offered the lower nutrient concentration diets. However, amino acid digestibility was either lower or there was a trend of lower (P<0.05 to P=0.09) values when control birds were compared to birds offered the high nutrient concentration diets, and comparative difference of Val and Met digestibility did not differ (P>0.05) between control and lower nutrient density diet fed birds. No difference (P>0.05) in digestibility of Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Ser, Thr, Tyr and Val was noted when control birds were compared to birds offered the high nutrient concentration diets.

Discussion

The analysed dietary concentration of crude protein (CP) were slightly lower and the values for K, Ca and Na concentration were higher than the calculated values, which was probably due to differences between the composition of the actual ingredients that were used in the present study and the NRC (1994) values for the same ingredients. The relatively higher final body weight of the birds, when compared to breed standards, may be explained by the 'small pen' effect, e.g. a reduction in competition for, and closer proximity to, drinkers and feeders.

Water intake measurements

At moderate temperatures feed intake, or more specifically dry matter intake, is the main determinant of the daily water requirement of poultry (Pond *et al*., 1995). However water intake and the ratio of water to food intake are increased by high dietary mineral and protein concentrations (Fuller *et al*., 2004). In order to maintain water balance, water intake must exactly counterbalance the water lost from the body as well as water stored in new growth therefore any over consumption from the requirement can lead to higher than normal water excretion. Since the dietary concentration of nutrients other than CP and AME were kept similar in all dietary treatments, however, NDF content changed significantly due to feed formulation constraints in the lower nutrient density diets, therefore, higher feed intake resulted in a higher mineral and NDF intake, which are known to increase water intake and excretion in poultry (Van der Klis *et al.*, 1995). Therefore as expected higher feed intake (FI) in the present study in birds fed on lower nutrient density diets resulted in higher water intake (WI) which then resulted in poor litter quality.

Feed intake and feed composition can affect metabolism and utilisation of individual amino acids which then can affect normal gut functioning and can impair absorption of other nutrients. Certain dietary factors such as fibre, lignins, tannins and lectins can influence threonine availability to the animal. It has been shown in the literature that threonine deficiency caused by either inadequate dietary supply or due to

factors mentioned above can result in increased excretion of mucins and abrasion leading to severe diarrhoea in pigs (Law *et al.*, 2007). Higher level of dietary NDF in poor nutrient density fed birds of present study could have resulted in poor absorption of nutrients across GIT, hence resulted in higher retention within digesta. In the present study lower amino acid digestibility in diets where nutrient density was lowest therefore, indicates that the dietary NDF content in diets formulated with lower nutrient density might have been the cause of lower amino acid digestibility and imbalance. An amino acid imbalance is highly likely to make things worse when compared with a well-balanced amino acid profile (D'Mello, 1993; D'Mello, 1994; Moran and Stilborn, 1996).Symptoms of imbalance or deficiency of linoleic acid in the domestic fowl include retarded growth, increased water consumption (Stevens, 2004). Higher NDF intake in birds fed with lower nutrient density diets in the present study created a severe imbalance of amino acids causing a reduction in protein utilisation and a lower FCE. Fibre itself is responsible for decreased protein digestibility in pigs, with water retention capacity being shown to increase ileal protein losses (Larsen *et al.*, 1993). It has been reported by Fairclough*et al*. (1980) that free amino acids exert more osmotic pressure than peptides, and free amino acids may in some cases be utilized even less efficiently than protein-bound amino acids (Boisen, 2003). Therefore, this situation could lead to excretion of water more than normal through excreta as reported in the present study. Diarrhoea can affect the availability of other amino acids (e.g. methionine) required for gut function and metabolism. For example, threonine is regarded as crucial for normal gut structure and function so its requirement is quite high. Pigs can use almost 60% of their threonine intake for gut development and functioning (Stoll *et al.*, 1998). Since threonine is required for gastrointestinal secretions (mucin) that protect mucosa from digestive proteases, dehydration, microbial and parasitic invasion and therefore, believed to play an important role in development and normal functioning of the gut (Bertolo *et al.*, 1998; Stoll *et al.*, 1998). Likewise any imbalance or improper supply of other amino acids such as leucine can affect gut functioning and structure. Adequate arginine intake is crucial for normal metabolic function in pigs and any deficiency can result in increased plasma ammonia concentration leading to metabolic disturbance (hyperammonemia) (Urschel *et al.*, 2007). These problems can be addressed by dietary supplementation of arginine (Zhan *et al.*, 2008). As it is required for the synthesis of protein, urea, nitric oxide and other metabolites and any inadequate supply for one or the other reasons can change the priority of its usage. This can result in higher concentration of ammonia in the plasma which is toxic and required more water for excretion. It is also documented in the literature that higher feed and mineral intake can depress DMD (Koreleski *et al.*, 2010) and amino acid absorption.

Further to amino acid imbalance and digestibility association with litter quality problems, undigested starch and protein favour proliferation of coliform bacteria in pigs (Jeaurond *et al.*, 2008). However, fibre can reverse the ratio of coliform bacteria to other beneficial bacteria (lactobacilli) and can reduce ammonia contents in GIT (Bikker *et al.*, 2006). But it is worth noting that source of fibre can produce different affects as fibre from wheat bran provides intermediate results.

Goldstein and Skadhauge (2000) highlighted that lower protein fed birds when had limited dietary energy available can have relatively higher quantity of nitrogen excreted in forms other than uric acid it is just to conserve energy. These forms e.g. urea and ammonia are osmotically active and require alot of water to be excreted. The lower dietary energy and its relationship with higher amino acids being oxidsed to be used as energy source were explained (Church, 1991; Pfeiffer, 1995; Musharaf and Latshaw, 1999) highlighting the fact that it is not the absolute dietary CP but the ratio between ME and CP is perhaps more important when a control on litter moisture and nitrogen is to be ensured. Caution is therefore necessary in reaching any conclusions when evaluating studies referring to relationship of dietary CP with litter moisture contents.

Litter quality associated parameters

An increase in nutrient density resulted in a reduction in the litter moisture (LM) content and this relationship suggested that the optimum dietary nutrient density for reduced LM does not match with the determined optimal density for bird growth. Therefore, the higher LM content reported in this study could have been the reflection of higher nutrient retention in digesta possibly due to poor DMD, OMD, amino acid digestibilities and presence of higher NDF content, when birds were fed lowest level of dietary energy and protein concentrations. However, present findings differ to some extent from findings reported by Khajali and Moghaddam, (2006) that there was no effect of lower dietary crude protein concentration on litter moisture content. However, they are in agreement with present findings of reduction in nitrogen excretion when birds were fed lower dietary protein concentration.

In terms of nitrogen excretion by the bird and a reduction in the litter NH_3 concentration these results are in line with previous findings of different studies which reported that a reduction in dietary protein content can help control nitrogen excretion and NH_3 emission from poultry litter (Jacob *et al.*, 1994; Moran and Stilborn, 1996; Ferguson *et al.*, 1998; Hussein *et al.*, 2001; Bregendahl *et al.*, 2002; Rezaei *et al.*, 2004; Si *et al.*, 2004). Uric acid is the end product of protein degradation in avian species and is a direct measure of protein catabolism in birds. Some researchers reported a decrease in uric acid concentration in the blood when lower protein diets were fed to broilers (Rosebrough *et al.*, 1996; Collin *et al.*, 2003). Different researches (Cheng *et al.*, 1997; Aletor *et al.*, 2000; Swennen *et al.*, 2004; Swennen *et al.*, 2005; Swennen*et al*. 2006) have reported that birds have mechanism to reduce amino acid oxidation as a sparing mechanism which therefore, is the reason of lower plasma uric acid level. Therefore, probable reason of this lower litter NH_3 content was

due to the lower uric acid excretion by the birds fed on lower nutrient density diets.

Leg health parameters

Increasing litter score (reflecting deterioration in litter quality) had a positive correlation with WI however, the negative correlation of WI with hock burn scores (HBS) may appear contrary to previous findings (Mayne *et al.*, 2007), because it might be expected that high water intake would result in poor litter quality or high LM with a resulting increase in contact dermatitis. The reduced litter moisture and lower litter scores were achieved with an increase in nutrient density which is in agreement with the findings of Kenny *et al*. (2010). However this improvement in litter quality did not correspond with the incidence of HB or FPD. The higher incidences of HB were associated with birds fed the higher nutrient density diet, in agreement with the findings of Bilgili*et al*. (2006). The positive correlation of HB with litter NH_3 indicates that perhaps litter chemical properties are important contributor in skin damage and litter moisture may only aggravate the damage by making skin more prone to these damages. Therefore, present findings suggested that it may be the litter NH_3 and pH which has a much greater effect on incidence of hock burn than litter moisture content alone. Therefore, in terms of HBS it was notable that increases in litter moisture were not associated with increased HBS. It is likely that the cause of the higher HBS in groups fed higher nutrient density diets was primarily litter NH_3. Unlike Ekstrand*et al*. (1997) and (1998) litter moisture was the main cause of footpad dermatitis (FPD). However, Dawkins *et al*. (2004) reported that a combination of litter moisture and ammonia was associated with poor health and correlated with 'dirty foot pads'. Berg (2004) also noted that HB lesions are commonly caused by a combination of moisture, high ammonia content, and other unspecified chemical factors in the litter. There is another possible reason for higher incidences of HB in birds fed the higher nutrient density diets. These birds may spend less time standing for feed and therefore, spend more time sitting on the litter. Haslam*et al*. (2007) reported that factors which increase bird weight or which are related to reduced litter quality, tend to increase hock burn.

Although litter moisture increased with age in this study there was a reduction in the HBS as well as FPDS which highlights that it is not litter moisture alone that can cause skin damage. These findings agree with the findings of Bilgili*et al*. (2006) who reported that the proportion of birds with footpad dermatitis tended to increase until 49 days of age after which they started to decline. So it is possible that older birds may become less susceptible to litter moisture damage (Mayne*et al*., 2007).

The findings in this study contrast with those of Mayne*et al*. (2007), who reported that litter moisture was the cause of FPD in turkeys. Increased litter moisture not associated with more incidences of FPD although these findings may be consistent with those of Dawkins *et al*. (2004) who concluded that both litter moisture and NH_3 are required to predispose birds to FPD rather than litter moisture alone.

Growth performance, dietary nutrient intake and utilisation

It is well documented that dietary composition and the ratios between macronutrients have a major impact on performance and body composition of chickens (Macleod, 1990; Macleod, 1992; Nieto *et al*., 1997; Collin *et al.*, 2003). In the present study birds fed on lower nutrient density had lower crude protein digestibility (CPD) as well as lower feed conversion efficiency (FCE) and protein efficiency ratio (PER) which are consistent with previous reports. For example, some studies have reported a negative effect on feed conversion ratio of lower crude protein concentration even when supplemented with synthetic amino acids (Moran and Stilborn, 1996; Ferguson *et al.*, 1998; Neto *et al.*, 2000). Layer birds eat to meet their energy requirement, so physical capacity and energy content can affect both feed intake (Morris, 1968; Golian and Maurice, 1992; Leeson *et al.*, 1993). Study of Huang *et al*. (2009), the present findings suggest that meat producing birds also try to compensate for any energy deficiency by increasing their feed intake when fed a lower nutrient density diet however, in this study, they were not able to match the similar weight gain as recore recorded in birds fed with higher nutrient density diets. The lower weight gain and poor feed conversion efficiency in the present study in birds fed on lower nutrient density was consistent with Hidalgo *et al*. (2004) who reported the same when broilers were fed diets with suboptimal levels of energy and crude protein while maintaining ME:CP. Farrell *et al*. (1973) and Farrell (1974) suggested that there is an optimum energy concentration in the diet beyond which the performance of birds does not appear to improve and that in some cases, it may actually deteriorate. The present findings agree with this conclusion only during the last growth phase (16-20 weeks) where maximum weight gain was recorded when birds fed with diet contain 100% nutrient density compared to either of the lower or higher nutrient density diet fed birds.

Others reported a reduced growth performance with a reduction of as little as 30g/kg dietary crude protein concentration even when the diet was supplemented with synthetic amino acids (Fancher and Jensen, 1989a; Fancher and Jensen, 1989b; Fancher and Jensen, 1989c; Pinchasov *et al.*, 1990; Colnago *et al.*, 1991; Kerr and Kidd, 1999; Aletor *et al.*, 2000; Waldroup, 2000; Bregendahl *et al.*, 2002). Whereas Aletor*et al*. (2000) reported improved protein efficiency ratio with lower dietary crude protein concentration because dietary protein is preferentially used for protein deposition. However, other studies also indicated the importance of dietary energy concentration along with CP as they reported poor protein deposition in the carcass in case the energy availability becomes limiting (Macleod, 1990; Musharaf and Latshaw, 1999).

Overall decrease in FCE, PER and an increase in feed intake (FI) with age in the present findings can be best explained by the fact that birds are able to retain more protein at younger age and with the age this ability

decrease and they retain more fat. Fat contains more energy than protein and gaining body fat require more feed intake to be converted to less body growth compared to protein.

The experimental diets were formulated to contain graded levels of dietary energy and protein concentrations, because, it was hypothesised, would affect feed and water intake and hence litter quality and would allow test of their response to different dietary concentrations. However, the overall changes in growth performance parameters were expected, i.e. most of the dietary energy and protein concentrations were beyond those used in commercial practice, therefore, they are not further discussed here.

The higher energy efficiency ratio (EER) in birds fed lower nutrient density diets seems to be at variance from the FCE and PER results. However, this can be explained by the uric acid excretion values of birds fed lower nutrient density diets being lower than for those birds fed on higher nutrient density diets. Uric acid formation and excretion is a process that requires significant energy. Therefore, birds fed on higher nutrient density diets use energy on uric acid excretion, hence had lower EER values. The present findings agree with the findings of Skinner *et al*. (1992) who reported that an increase in dietary nutrient density resulted in depressed energy efficiency.

Poor nutrient utilisation i.e. CPD, dry matter (DM), organic matter (OM) and amino acid digestibilities in birds fed lower nutrient density diets in the present study could be explained by the presence of higher concentration of neutral detergent fibre (NDF) in the diets formulated to present lower nutrient concentrations. The proportion of cellulose and lignin in the crude fibre fraction also determines the digestibility of crude fibre or its solubility in the intestine. AWT (2005) report by-products of cereal processing such as wheat bran to be particularly high in fibre while soybean meal (especially high protein grades) bring little fibre into the formulation (e.g. pentosans i.e. arbinose and xylose etc. wheat bran 250 g vs. 35 g/kg DM in soybean meal). Since fibre has no direct nutritive benefit in poultry nutrition the high cellulose and lignin concentrations as result of formulation constraint to add wheat bran could have resulted in reduced nutrient digestibility.

Conclusion

The present experiment has shown that an increase in the concentration of dietary crude protein (CP) and apparent metabolisable energy (AME) can reduce water intake (WI), decreasing moisture content in the litter and thereby reduce the litter score (indicating improved overall litter quality).However, the incidence of hock burn increased with the high nutrient density diets, suggesting that factors other than the litter moisture alone may contribute the occurrence of leg health (defined in this study as FPD and HB) problems in turkey production.

The incidence of hock burn (HB) was associated with litter NH_3. Since CP intake was related to litter NH_3 concentration, then modifying the CP intake by altering the calorie to CP ratio may be one way of controlling HB by dietary manipulation.

It is perhaps important to report that good litter score (based on physical appearance) was not related to litter NH_3 and pH therefore litter score per se is of limited or no value in terms of lowering HB incidences in turkey production.

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Competing Interests

The authors declare that they have no competing interests.

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