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Original article

Effect of dietary protein level, amino acid balance and feeding level on growth, gastrointestinal tract, and mucosal structure of the small intestine in broiler chickens

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Abstract — In a factorial experiment, two series of feeds containing excess dietary protein, differing in amino acid balance (i.e. balanced (BPS) and unbalanced (UPS) amino acid mixture), and with a range of protein contents (400, 300 and 200 g CP·kg^{-1}) at the same energy content of 13 MJ AME·kg^{-1} were offered at two levels of feeding (ad libitum or 0.75 of ad libitum intake) to 4320 broiler chickens between 10 and 24 days of age. Growth rate was significantly lowered by feed restriction. There was also a significant ($P < 0.001$) effect of dietary protein on the combined weight of the proventriculus and gizzard but only for the birds on the restricted feeding regime. Relative pancreatic weight increased ($P < 0.001$) with an increase in dietary protein level for the birds fed restricted amounts of BPS. The crypt depth of chicks on the ad libitum feeding regime was higher ($P < 0.01$) for the chicks on the BPS than for those on the UPS diet. The protein content of the jejunal mucosa was higher ($P < 0.001$) for birds fed ad libitum on the UPS diet than on the BPS diet. Daily feed allocation had a significant ($P < 0.01$) effect on jejunal protein content in birds that received the BPS diet, this being reduced in birds on restricted feeding. Maltase ($P < 0.001$) and sucrase ($P < 0.01$) activities were significantly reduced in chicks offered ad libitum access to the UPS diet. At high dietary CP, the specific activity of alkaline phosphatase was lower ($P < 0.001$) in chicks on the UPS diet than in those fed the BPS diet.

broiler / excess protein / feed restriction / enzyme / viscera

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Résumé — Effets de la concentration en protéines, de l'équilibre en acides aminés et du niveau d'ingestion de l'aliment sur la croissance, le développement du tube digestif et la structure de la muqueuse jéjunale chez le poulet de chair. Six aliments isoénergétiques (13 MJ EMa·kg^{-1}) selon le plan factoriel : 3 concentrations en protéine brute (400, 300 et 200 PB g·kg^{-1}) × 2 équilibres en acides aminés (BPS = équilibré, UPS = déséquilibré par rapport aux besoins), ont été offerts à deux niveaux d'ingestion (ad libitum ou 75 % de l'ad libitum) à 4320 poulets de chair entre 10 et 24 jours d'âge. La vitesse de croissance était significativement réduite par la restriction alimentaire. Un effet du taux protéique sur le poids combiné du proventricule et du gésier était significatif ($P < 0,001$) mais uniquement chez les poulets restreints. Le poids relatif du pancréas augmentait ($P < 0,001$) avec la concentration du régime protéines BPS chez les poulets restreints. La profondeur des cryptes des villi de l'intestin était plus profonde chez les poulets nourris ad libitum avec les régimes BPS par rapport aux régimes UPS ($P < 0,01$). La concentration en protéine de la muqueuse jéjunale était supérieure chez les poulets consommant ad libitum les régimes UPS par rapport à ceux ingérant les régimes BPS ($P < 0,001$). La restriction alimentaire réduisait également la teneur en protéine du jéjunum ($P < 0,01$) chez les poulets consommant les régimes BPS. Les activités maltase ($P < 0,001$) et sucrase ($P < 0,01$) étaient significativement inférieures chez les poulets consommant ad libitum les régimes UPS. A haute teneur en protéine, l'activité spécifique de la phosphatase alcaline était réduite ($P < 0,001$) chez les poulets consommant les régimes UPS par rapport à ceux recevant les régimes BPS.

poulet de chair / excès de protéine / restriction alimentaire / enzyme / tube digestif

1. INTRODUCTION

The synthesis of protein, i.e. protein deposition in broiler chickens, is a process that requires a large amount of energy and is to some extent dependent on bird-related factors such as the development of the gastrointestinal tract (GIT) [30]. Apart from the commonly assessed effects of the energy to protein ratio (E:P ratio) on the biological performance of broiler chickens, the study of causal connections at the GIT/organ level has been largely ignored or underestimated. The efficiency of utilisation of dietary nutrients partly depends on the development of the gastrointestinal tract. Such development can be assessed through measurements of the crypt, a region in which new intestinal cells are formed; villus height and surface area, to determine the area available for digestion/absorption, and the activities of membrane-bound digestive enzymes of the small intestine. Such assessment has been routinely done in poultry [2, 17, 18, 38] but the results have not been adequately related to the physical environment of the bird.

The maximum E:P ratio at which the potential growth rate of the bird is met is likely to depend on the excess dietary protein (and hence amino acids, AA), dietary energy level and the bird's genetic potential. Biological performance is thus regulated by the dietary nutrient to energy balance through the following: changes in feed intake, absorption of balanced amounts of nutrients and metabolism of amino acids required for protein accretion [3, 40]. Nutrient processing by the GIT determines the amount of nutrient that is available to the internal tissues for metabolism. The GIT uses some of the digested nutrients for self-renewal and the efficiency of nutrient supply to the internal tissues is dependent on dietary factors, including the E:P ratios. The preliminary work on this relationship carried out by our research group (unpublished) demonstrated clear effects of excess dietary protein on the productive efficiency of broiler chickens. Some of the effects were partly explained by changes in the pattern and rate of development of the GIT, as recently reported [17, 18].

There is a dearth of reports linking the effects of dietary nutrients and especially that of energy and protein to the development of the GIT of poultry. Dietary protein has been associated with the regulation of

insulin-like growth factors (IGF) and somatotropin and thus, body growth and fat deposition in broiler chickens [6, 21]. Kita et al. [20] also reported on the effects of feed restriction on plasma IGF concentrations in broiler chickens. Feed restriction for 4–7 days was found to reduce plasma IGF, as did the consumption of low-protein diets.

In practice, higher-than-normal protein contents may be offered to broiler chickens when poor quality proteins are available but amino acid supplements are expensive and unavailable. This is done in order to provide a minimal level of essential amino acids (EAA) in the diet. This strategy invariably results in large amounts of unutilised AA that are not needed for protein synthesis and ultimately leads to suboptimal bird performance [39]. There is therefore a problem when high levels of protein, in excess of the broiler chicken's needs, are fed. A method of measuring this was to produce a range of feeds varying in metabolisable energy to digestible crude protein (ME:CP) ratio and protein quality to see whether the chicks' performance, GIT and mucosal structure of the small intestine were affected.

Thus the aim of the present study was:

(a) to confirm the hypothesis that GIT function is dependent on dietary energy and protein content, protein quality and daily food allocation, and

(b) to secure information on the effects of these factors on visceral organ development, mucosal structure, and digestive function associated with digestion and nutrient absorption.

2. MATERIALS AND METHODS

2.1. Experimental design

Two series of feeds differing in amino acid balance (AAB) and covering a range of three crude protein levels (i.e. 200, 300 and 400 g CP·kg^{-1}) at a constant energy content of 13 MJ ME·kg^{-1}, were offered at two daily food allocation (DFA: ad libitum or 0.75 of ad libitum) to male broiler chickens, 10 to 24 days of age (2 × 3 × 2 factorial) (Tab. I). It must be stressed that these diets were designed to decrease the ME:DCP ratio below the critical value, such that the efficiency of protein (e_p) utilisation was compromised. The amino acid contents of the two diets are shown in Table II. Seventy-two single cages were available for the study, so the treatments were replicated six times.

2.2. Birds and housing

Four-thousand three-hundred-and-twenty male broiler chickens of uniform size were used for the study. During the pre-test period (0–9 days post-hatching) the chicks received a standard commercial starter feed (240 g crude protein·kg^{-1}) ad libitum. Day-old chicks were weighed and placed in groups of ten in single-tier battery cages in the experimental unit, to accustom them to the facilities. At 10 days of age, the birds (215.8 ±15.3 g) were randomly assigned to one of the 12 feeding treatments, such that the average starting weight and weight range were similar for each treatment. The experimental birds were given ad libitum access to water and continuous artificial lighting until the end of the experiment. The ambient temperature was gradually decreased from 30 ºC to 24 ºC over the experimental period of 10–24 days of age.

2.3. Experimental diets

Two dietary protein series were used in the study, one being based on a balanced (BPS), and the other being based on an unbalanced (UPS) amino acid mixture (Tab. I). These were each fed at three protein levels and at two daily food allocations. The BPS consisted mainly of soybean, canola and fishmeal, whereas the UPS was

Table I. Ingredient composition and nutrient contents of the balanced (BPS), unbalanced (UPS) protein series and the protein free diets used in the experiment.

Raw ingredient (g·kg^{-1})	BPS	UPS	Protein free
Maize	173.92	109.70	-
Fishmeal 65	83.28	-	-
Maize gluten	50.00	360.00	-
Soya protein (66%)	50.00	-	-
Soybean oil cake	529.53	-	-
Lupin	-	451.10	-
Sunflower oil	75.62	40.10	60.00
Starch	-	-	333.90
Sugar	-	-	333.90
MCP[1]	9.33	16.10	20.00
Vitamins + Minerals	2.50	2.50	2.50
Salt	-	2.21	25.00
Sodium bicarbonate	1.61	1.31	1.30
Limestone	9.33	16.10	18.33
Filler (plaster sand)	-	-	176.34
Nutrients			
Dry matter	904.95	905.9	840.6
Crude protein	400.00	400.0	-
AMEn (MJ·kg^{-1})	13.00	13.00	13.00
Fat	100.00	103.20	200.00
Crude fibre	26.73	58.90	-
Ash	51.54	26.10	13.00
Calcium	10.00	10.00	10.00
Phosphorus	5.00	5.00	5.00
Sodium	1.50	1.50	1.50
Chloride	1.80	1.80	1.80

[1] Monocalcium phosphate.

based on maize gluten and lupins. The two basal diets were formulated to contain 13 MJ AME·kg^{-1} and 400 g CP·kg^{-1}. In the formulation of the BPS diet, total essential AA were minimised. The amino acid specifications applied were those suggested by the EFG broiler growth model [9] for broilers aged 10 to 24 d and are similar to the specifications of NRC [29]. The mean lysine requirement over this period is 12 g lysine·kg^{-1}. The basis of these requirements is that a broiler will be depositing body and feather protein, of known amino acid composition at a rate determined by its potential

Table II. The amino acid composition ($g \cdot kg^{-1}$) of the balanced (BPS) and unbalanced (UPS) protein sources used in the experiment.

Amino acid	BPS	UPS
Lysine	24.49 (100)	12.48 (100)
Methionine	15.59 (63)	6.73 (58)
Methionine+cystine	10.65 (43)	13.20 (106)
Threonine	15.28 (62)	13.98 (112)
Tryptophan	4.29 (18)	2.52 (20)
Arginine	26.71 (109)	26.27 (210)
Histidine	10.44 (43)	8.61 (69)
Isoleucine	18.92 (77)	17.82 (143)
Leucine	32.29 (132)	50.00 (400)
Phenylalanine	19.15 (78)	20.97 (168)
Valine	20.25 (83)	18.38 (147)

The amounts of amino acids relative to lysine are shown in brackets.

growth rate. The lysine concentrations ($g \cdot kg^{-1}$) used in the BPS and UPS diets had a range of 12.5 to 24.9 and 6.0 to 12.1, respectively.

The lysine (lys):CP ratio of the diets in the BPS group ranged between 0.061 and 0.062 (g lys·kg^{-1} CP). The lys:CP ratios in the UPS group with a range of 0.015 to 0.031 g lys·kg^{-1} CP were lower than the minimum (i.e. 0.057) recommended for maximum growth by Morris et al. [26]. The digestibility of crude protein (dcp), which is the amount of total crude protein available to the bird as a result of the chemical nature of the raw ingredients of the particular feed were determined from the European Table of Energy Values for Poultry Feedstuffs [10]. The dcp value for the mixed feed was calculated by determining the proportion of digestible crude protein to total crude protein of the various raw materials relative to their proportion in the diet. The range of the dcp for the BPS and UPS series were 0.39 to 0.79 and 0.42 to 0.83, respectively. The proportions of total digested protein, which can be incorporated into body protein, 'V' for the BPS and UPS were 1 and 0.52 to 1, respectively.

The high protein summit diets were formulated first. These were then blended in appropriate proportions with protein-free sources of energy, minerals and vitamins (Tab. I), to obtain two series of diets with a similar amino acid balance in each series but differing in protein content. This procedure, expounded by Gous and Morris [12] for estimating the response to varying intakes of an amino acid is called the diet dilution technique. To obtain a 300 or 200 g CP·kg^{-1} diet, a blend of the summit diets (i.e. 400 g CP·kg^{-1}) to protein free mixture in the ratio of 3:1 and 1:1, respectively was used. The crude protein contents within each series were 400, 300 and 200 g CP·kg^{-1}, respectively, all with the same energy content of 13 MJ AME·kg^{-1}, resulting in three E:P ratios, viz.: 32.5, 43.3 and 65.0 MJ ME·kg^{-1}.

2.4. Dietary treatments

2.4.1. Ad libitum treatments

Birds assigned to these treatments were given free and continuous access to one of the 6 dietary treatments. Feed consumption

was measured daily by weighing the food at the start and end of each 24-hour period.

2.4.2. Restricted treatments

Birds designated to these treatments were restricted to 0.75 of the average consumption of the respective ad libitum treatments over the previous day. The allocated feed was divided into two portions, with the first being given in the morning at 7 a.m. and the other in the afternoon at 2 p.m. The same levels of restriction were applied for each of the two protein series. Birds allocated food at 0.75 of the previous ad libitum will become less restricted due to the increasing difference in weight between the ad libitum and the restricted fed birds.

2.5. Bird management procedure

Birds were fed a mash feed mixture twice daily and weighed once weekly. Feed was fed in a uniform mash mixture and there was no selection of particles by the ad libitum fed birds. Fresh feed was weighed and offered to all the birds twice daily to prevent wastage. At the conclusion of the experiment, all birds and the remaining feed were weighed.

2.6. Sample collection

At the end of the experimental period, one bird per cage, selected at random, was slaughtered through asphyxiation with CO_2 and dissected. The birds were killed 13 hours after their last meal. The joint weights of the proventriculus and gizzard as well as the weight of the small intestine were recorded. The pancreas, liver and spleen were also weighed. Tissue samples were taken from the proximal region of the jejunum and flushed with ice-cold saline. Some of these samples were snap-frozen and used for the digestive enzyme assay. A subsample was fixed in neutral buffered formalin and used to assess the morphometry of the intestinal mucosa.

2.7. Histology

Tissue slices were dehydrated manually and embedded in paraffin wax. Sections were cut from the waxed tissue on a Leitz 1512 microtome (Ernst Leitz Westlar GmBH, Austria) and were cleared of wrinkles by floating on warm water (45–50 ^{o}C) prior to mounting on 10% poly-L-lysine coated slides. The slides were stained by Lilee Meyer haematoxylin, counter-stained with eosin yellow and mounted in a DePeX medium.

The slides were viewed on an Olympus BH-2 microscope and digitised using a video image software, Video Pro (Leading Edge, Bedford Park, South Australia). The images were viewed (optical lens No. 4) to measure the crypt depth, villus width at the crypt-villus junction, villus height and villus apical width. Apparent villus surface area was estimated through trigonometry [17]. Fifteen villi were assessed per sample.

2.8. Measurement of the digestive enzymes

The intestinal tissue homogenate was prepared as described by Shirazi-Beechey et al. [33]. The tissue was cut into an ice-cold buffer (100 mM mannitol, 2 mM Tris/HEPES, pH 7.1) and the mucosa was then stripped into the buffer using a swirl mixer at high speed for one minute. The mixture was homogenised at medium speed for thirty seconds. Sub-samples of the homogenate were taken into Eppendorf tubes, frozen in liquid nitrogen and stored in a deep freezer (–20 ^{o}C) for enzyme analysis.

Enzyme assays were conducted on fixed substrate concentrations established in studies on other species and previously standardised for poultry [18]. Biochemical assays were conducted for maltase (EC. 3.2.1.20), sucrase (EC. 3.2.1.26) and alkaline phosphatase (AP, EC. 3.1.3.1).

The specific activities of enzymes were measured according to methods previously described for other species [8, 15, 24]. The assays were, however, conducted at a temperature of 39 °C. The protein content of the jejunal mucosa was measured according to the method described by Bradford [4].

2.9. Statistical design and analysis

The data collected were analysed by the general linear model (GLM) of Minitab [25] as a 2 × 3 × 2 factorial design. Mean values were compared with the F-test and least significant difference and were deemed to be significant at $P \leq 0.05$.

3. RESULTS

3.1. Feed intake and utilisation

Feed intake was significantly influenced ($P < 0.001$) by protein balance, dietary crude protein content and feeding level as well as the interaction between protein balance and crude protein content (Tab. III). At a dietary protein content of 200 g·kg^{-1}, the birds tended to consume less feed on the unbalanced diets than on the balanced diets. This effect was significant for the birds on the restricted feed intake, containing 300 and 400 g CP·kg^{-1}. Feed intake declined ($P < 0.001$) with an increase in dietary protein content for the birds on the balanced diets fed ad libitum or restricted. There was no significant effect of varying crude protein content on feed intake on the UPS diets when fed ad libitum or restricted.

At both feeding levels, there was a reduction ($P < 0.001$) in the body weight gain of birds on the unbalanced diets, at protein contents of 200 and 300 g CP·kg^{-1} diet. At the 400 g CP level, this effect was observed only on the balanced diets. Body weight gain declined ($P < 0.001$) with an increase in dietary crude protein for the birds on the balanced diets; the reverse was the case for those on the unbalanced diets. The interaction between protein balance and crude protein and that between protein balance and feed level on body weight gain were significant ($P < 0.001$).

For birds reared on the 200 and 300 g CP·kg^{-1} diets, FCE was poorer ($P < 0.001$) for those on the unbalanced protein diets than those on the balanced diets. For the 400 g CP·kg^{-1} diets, this effect was noticeable only in chicks that were fed ad libitum. FCE also increased ($P < 0.001$) with an increasing dietary CP content although the trend for the chicks on the balanced protein diet, on a restricted regime was not consistent. FCE was also influenced ($P < 0.01$) by the interactions between crude protein and feeding level, protein balance and feeding level as well as between protein balance and crude protein content ($P < 0.001$). As expected, the birds on restricted feeding gained significantly less weight ($P < 0.001$) and had a poorer FCE than those on the ad libitum feeding regime.

3.2. Visceral organ weight

The weight of the visceral organs from birds on the various diets is shown in Table IV. For chicks on the diets containing 300 g·kg^{-1}, the combined weight of the proventriculus and gizzard on the balanced diet was significantly lower ($P < 0.001$) than that of the chicks on the unbalanced diet. There was also a significant ($P < 0.001$) effect of dietary protein but only for the birds fed the restricted diets. The interaction between protein balance and dietary crude protein content was significant ($P < 0.05$). The weight of the small intestine was influenced ($P < 0.001$) by a variation in feeding level ($P < 0.001$) and the interactions between crude protein and feeding level ($P < 0.05$) in chicks on the unbalanced diets containing 200 g CP·kg^{-1}. The weight of the pancreas was lowest ($P < 0.001$) in chicks on the unbalanced diets, containing 400 g CP·kg^{-1}. In chicks fed

Table III. The effect of excess dietary protein, amino acid balance, daily food allocation on feed intake, growth and FCE in broilers between 10 and 24 days of age.

Protein balance (AAB)	CP ($g \cdot kg^{-1}$)	Daily food allocation (DFA)	Feed intake ($g \cdot bird^{-1} \cdot d^{-1}$)	Weight gain ($g \cdot d^{-1}$)	FCE (g weight gain$\cdot kg^{-1}$ feed)
BPS	200	1	64.9^{a}	29.8^{ab}	459.0^{bc}
		0.75	48.7^{cd}	19.1^{de}	391.9^{de}
UPS	200	1	48.6^{cd}	17.0^{ef}	350.9^{e}
		0.75	36.4^{fg}	11.2^{g}	306.7^{g}
BPS	300	1	58.1^{b}	30.9^{a}	531.2^{a}
		0.75	43.6^{e}	18.7^{de}	428.7^{cd}
UPS	300	1	53.4^{bcd}	22.7^{c}	424.9^{cd}
		0.75	40.1^{g}	13.9^{f}	347.4^{f}
BPS	400	1	50.9^{cd}	26.9^{b}	528.7^{a}
		0.75	38.2^{fg}	14.6^{f}	381.2^{e}
UPS	400	1	47.4^{de}	22.7^{c}	466.0^{b}
		0.75	35.6^{g}	13.8^{f}	390.8^{de}
		SEM	1.73	1.02	15.05
Source of variation					
AAB			0.0001	0.0001	0.0001
CP			0.0001	0.0001	0.0001
DFA			0.0001	0.0001	0.0001
AAB × CP			0.0001	0.0001	0.0001
CP × DFA			0.156	0.0001	0.002
AAB × DFA			0.497	0.067	0.003
AAB × CP × DFA			0.594	0.787	0.197

Mean values in the same column with unlike superscripts are significantly different at the levels indicated. NS: not significant. AAB: amino acid balance; DFA: daily food allocation (proportion of ad libitum intake); BPS: balanced protein series; UPS: unbalanced protein series; CP: dietary crude protein.

restricted amounts of the balanced protein diets, pancreatic weight increased ($P < 0.001$) with an increase in dietary protein level. The weight of the spleen was influenced ($P < 0.001$) by the feeding level for chicks on the 200 g CP$\cdot kg^{-1}$ diets. There was also a significant ($P < 0.05$) effect of the feeding level on the weight of the liver but this was not consistent.

3.3. Intestinal mucosal morphometry

The crypt depth of chicks on the ad libitum feeding regime was higher ($P < 0.01$) for those on the balanced diets than for those on the unbalanced diets (Tab. V). Crypt depth was also influenced ($P < 0.05$) by a variation in crude protein content within the UPS. Villus height was reduced

Table IV. The effect of excess dietary protein, amino acid balance, and daily food allocation on the weight (g per100 g body weight) of visceral organs in broilers between 10 and 24 days of age.

Protein balance	CP ($g \cdot kg^{-1}$)	DFA	Proventriculus + Gizzard	Small intestine	Pancreas	Spleen	Liver
BPS	200	1	4.2^{b}	4.5^{bc}	0.30^{bc}	0.14^{ab}	3.2^{b}
		0.75	4.2^{b}	5.3^{ab}	0.30^{bc}	0.21^{a}	3.9^{ab}
UPS	200	1	5.2^{b}	4.3^{c}	0.30^{bc}	0.09^{b}	3.3^{b}
		0.75	5.4^{b}	5.3^{ab}	0.40^{bc}	0.17^{a}	4.2^{ab}
BPS	300	1	4.1^{b}	4.5^{bc}	0.40^{bc}	0.12^{ab}	3.4^{b}
		0.75	4.9^{b}	5.2^{ab}	0.50^{ab}	0.13^{ab}	3.3^{ab}
UPS	300	1	5.3^{b}	4.7^{bc}	0.40^{bc}	0.11^{b}	3.6^{ab}
		0.75	5.9^{a}	5.2^{ab}	0.40^{bc}	0.17^{a}	4.0^{ab}
BPS	400	1	4.1^{b}	5.6^{a}	0.50^{ab}	0.14^{ab}	3.7^{ab}
		0.75	4.5^{b}	5.1^{ab}	0.60^{a}	0.22^{a}	4.3^{a}
UPS	400	1	4.3^{b}	4.8^{ab}	0.30^{c}	0.13^{ab}	3.9^{ab}
		0.75	4.3^{b}	4.9^{ab}	0.40^{bc}	0.16^{ab}	3.3^{b}
		SEM	0.48	0.34	0.046	0.033	0.35
Source of variation and level of probability							
AAB			0.0001	0.137	0.001	0.183	0.593
CP			0.001	0.099	0.0001	0.236	0.399
DFA			0.095	0.001	0.001	0.0001	0.031
AAB × CP			0.039	0.077	0.0001	0.189	0.052
CP × DFA			0.507	0.025	0.482	0.479	0.083
AAB × DFA			0.774	0.764	0.236	0.918	0.526
AAB × CP × DFA			0.822	0.828	0.477	0.407	0.052

Mean values in the same column with unlike superscripts are significantly different at the levels indicated. NS: not significant. AAB: amino acid balance; DFA: daily food allocation (proportion of ad libitum intake); BPS: balanced protein series; UPS: unbalanced protein series; CP: dietary crude protein.

($P < 0.001$) in the chicks on the unbalanced diets containing 200 g $CP \cdot kg^{-1}$. Although the apparent villus surface area was influenced ($P < 0.001$) by protein balance, there was no consistency between the diets varying in crude protein content or feeding level.

3.4. Activities of digestive enzymes in the jejunum

At higher protein contents (300 and 400 g $CP \cdot g^{-1}$ diet) and unrestricted feeding, the protein content of the jejunal mucosa was higher ($P < 0.001$) in chicks on the unbalanced than on the balanced diets (Tab. VI). Feeding level also had a significant ($P < 0.01$) effect on jejunal protein content in the birds that received balanced diets containing 200 or 400 g $CP \cdot kg^{-1}$, with mucosal protein being reduced in the birds on the restricted feeding. There were also significant interactions between AAB and CP level ($P < 0.01$), AAB and FL ($P < 0.01$)

Table V. The effect of excess dietary crude protein, amino acid balance, and daily food allocation on morphometry of the jejunal mucosa in broilers between 10 and 24 days of age.

Protein balance (AAB)	CP ($g \cdot kg^{-1}$)	DFA	Crypt depth (μm)	Villus height (μm)	Villus: crypt ratio	Villus surface area (mm^2)
BPS	200	1	558.9^{ab}	1251.6^{a}	2.2	0.28^{a}
		0.75	537.6^{ab}	1014.3^{ab}	1.9	0.25^{ab}
UPS	200	1	402.5^{c}	875.1^{b}	2.2	0.20^{ab}
		0.75	454.2^{b}	847.2^{b}	1.9	0.18^{b}
BPS	300	1	548.4^{ab}	1196.1^{ab}	2.2	0.27^{a}
		0.75	563.2^{ab}	1021.9^{ab}	1.8	0.26^{ab}
UPS	300	1	522.5^{abc}	1010.6^{ab}	2.0	0.21^{ab}
		0.75	464.4^{bc}	939.9^{ab}	2.1	0.19^{ab}
BPS	400	1	629.8^{a}	1144.5^{ab}	1.7	0.27^{a}
		0.75	523.3^{abc}	1047.6^{ab}	2.0	0.25^{ab}
UPS	400	1	580.1^{ab}	907.7^{ab}	1.6	0.24^{ab}
		0.75	534.1^{ab}	918.0^{ab}	1.7	0.22^{ab}
		SEM	121.47	298.00	0.65	0.078
Source of variation						
AAB			0.002	0.001	0.745	0.0001
CP			0.018	0.737	0.537	0.445
FL			0.178	0.063	0.084	0.143

Mean values in the same column with unlike superscripts are significantly different at the levels indicated. NS: not significant. Interaction terms were not significant and were therefore not included in the analytical model. AAB: amino acid balance; DFA: daily food allocation (proportion of ad libitum intake); BPS: balanced protein series; UPS: unbalanced protein series; CP: dietary crude protein.

and between all three factors ($P < 0.05$). The specific activity of maltase was lower ($P < 0.001$) in the chicks on the unbalanced diets but this was only observed for birds on the diets containing 400 g $CP \cdot kg^{-1}$ and on an ad libitum feeding regime. The interactions between AAB and FL and between all three factors were also significant ($P < 0.05$). For the diets with CP contents higher than 200 $g \cdot kg^{-1}$, fed ad libitum, the specific activity of sucrase was lower ($P < 0.01$) in birds on the unbalanced diets than it was for the balanced diets. There was also a reduction ($P < 0.05$) in sucrase activity with an increasing dietary CP content in birds fed ad libitum on the unbalanced diets. At high dietary CP, protein balance affected ($P < 0.001$) the specific activity of AP, this tending to be lower in chickens on the unbalanced diets than in those fed the balanced diets. There were also significant interactions between AAB and FL ($P < 0.001$) and between the three factors ($P < 0.05$).

4. DISCUSSION

It must be emphasised that there has been limited research on the effects of varying protein levels, protein quality and feed

Table VI. The effect of excess dietary crude protein, amino acid balance, daily food allocation on the mucosal protein content and activities of digestive enzymes in the jejunum in broilers between 10 and 24 days of age.

Protein balance (AAB)	CP ($g \cdot kg^{-1}$)	DFA	Protein[1]	Maltase[2]	Sucrase[3]	AP
BPS	200	1	126.2^{a}	2.2^{ab}	0.07^{ab}	1.2^{ab}
		0.75	92.4^{bc}	2.0^{ab}	0.06^{abc}	1.2^{ab}
UPS	200	1	115.7^{a}	1.9^{ab}	0.07^{a}	0.8^{b}
		0.75	98.9^{abc}	2.1^{ab}	0.05^{abc}	0.9^{b}
BPS	300	1	72.9^{bc}	2.3^{ab}	0.07^{a}	1.5^{a}
		0.75	95.4^{bc}	1.6^{ab}	0.04^{bc}	1.0^{ab}
UPS	300	1	164.9^{a}	1.1^{b}	0.04^{bc}	0.6^{b}
		0.75	98.8^{abc}	1.8^{ab}	0.04^{bc}	1.1^{ab}
BPS	400	1	64.9^{c}	2.5^{a}	0.06^{ab}	1.8^{a}
		0.75	63.1^{a}	2.7^{a}	0.06^{ab}	1.3^{ab}
UPS	400	1	165.2^{bc}	1.1^{b}	0.03^{c}	0.6^{b}
		0.75	96.6^{bc}	1.7^{ab}	0.04^{bc}	1.1^{ab}
		SEM	19.81	1.3	0.011	0.21
Source of variation						
AAB			0.0001	0.0001	0.008	0.0001
CP			0.446	0.191	0.029	0.253
DFA			0.002	0.371	0.066	0.949
AAB × CP			0.004	0.014	0.114	0.183
CP × DFA			0.783	0.493	0.312	0.979
AAB × DFA			0.007	0.028	0.161	0.001
AAB × CP × DFA			0.028	0.250	0.098	0.041

[1] $mg \cdot g^{-1}$ tissue; [2] μmole glucose·mg^{-1} protein·$minute^{-1}$; [3] μmole nitrophenol·mg^{-1} protein·$minute^{-1}$; AAB: amino acid balance; DFA: daily food allocation (proportion of ad libitum intake); BPS: balanced protein series; UPS: unbalanced protein series; CP: dietary crude protein.
Mean values in the same column with unlike superscripts are significantly different at the levels indicated; NS: not significant.

restriction on the development of the GIT of broiler chickens. The general response of poultry to feed supply and quality is much better documented [7, 37, 42] but the mechanisms underlying these effects are not yet fully understood. An understanding of the effects of these factors is made more difficult by the fact that they operate in synergy with each other rather than singly. The data on the effect of excess dietary protein, amino acid balance, and daily food allocation on the GIT and mucosal structure of the small intestine is new and may have some important conceptual implications. The poorer performance observed for birds fed diets of low energy to protein (E:P) ratios suggests that when surplus protein is fed, the energy content should also be increased to ensure

that sufficient energy is available for the efficient utilisation of the dietary protein.

The curtailment in response associated with the feeding of surplus protein exceeding the requirement for maximum growth has also been reported by Harper et al. [14], using free amino acids and Wethli et al. [39], with the feeding of high amounts of poor quality protein. The effect of excess dietary protein is important because the excess load of absorbed AA may exert an unbalancing effect. Lewis [23] suggested that such surpluses of AA have to be balanced with increases in the specified minimum concentrations for AA that may be present in an inadequate ratio in the diet. Specifying these requirements as a proportion of the protein and not as a proportion of the diet, ensures that if it is economically desirable to formulate diets with higher-than-normal protein contents, an upward adjustment is made to the minimum dietary AA (i.e. lysine) concentration [26]. The minimum lys:CP ratio for birds fed the BPS was therefore greater than the minimum recommended by Morris et al. [27], whereas the ratios for diets in the UPS were lower than the minimum levels recommended, accounting for the depression in growth rate of the birds fed these diets. At the metabolic level, some explanations for the poor performance and utilisation of excess dietary protein have also been advanced by Moundras et al. [28] and Morris et al. [27]. Moundras et al. [28] attributed the poor performance at high protein concentrations (i.e. 600 casein·kg^{-1} protein diet) to the depletion of some critical glucogenic AA such as threonine. There is also the decreased availability of certain AA that are associated with the control of protein intake.

The results of this study revealed many of the interactions between some of the dietary factors. For example, feed intake responded differently on account of amino acid balance (AAB) as well as feed supply and dietary protein content. Thus, feed intake was affected by AAB but only at a low dietary protein content. Similarly, the response to dietary protein content was only observed on the balanced diets fed ad libitum. These responses are difficult to explain but may be related to the concentrations of amino acids at the different levels of dietary crude protein and feeding level [7].

Weight gain was low on the two lowest dietary protein contents (200 and 300 g CP·kg^{-1} diet), regardless of the AAB or FL. The negative effects of restricted feed intake on body weight gain or growth have been extensively reported by previous researchers [11, 13, 19, 37]. Gonzales et al. [11] partly attributed this effect to reductions in thyroxine, IGF and growth hormone. The effects of low protein supplies have been ameliorated by supplementation with methionine and cysteine, as long as other indispensable amino acids are available in sufficient amounts [5]. In the present study, FCE increased with an increase in dietary protein content. This confirmed the reports by Zaghari et al. [41] who observed a positive effect of increasing dietary protein content on FCE and body weight. In a comparative study, Ramlah et al. [32] also reported the positive effects of mild feed restriction on FCE.

The differences in the weight of visceral organs may partly explain some of the underlying mechanisms in the overall response to dietary factors, such as those examined in the present study. In the current study, AAB had some effect on the weight of the proventriculus and gizzard while dietary protein content had a positive effect on the weight of the pancreas under a restricted feeding regime. The increase in the weight of the pancreas in chicks that were reared on high protein diets may be due to the need for increased secretion of pancreatic proteases that target protein. In a previous study, Leeson and Zubair [22] observed an increase in the weight of the liver in chickens that were fed a high protein diet. There are controversies with regards to the

effects of feed restrictions on the development of visceral organs [31, 32]. Generally, the organs associated with nutrient derivation may not respond to poor nutrition due to the fact that such organs are preferentially developed in early life [17, 37]. In some instances, feed restriction has been observed to lead to an increase in the weights of visceral organs [42], probably as a result of birds trying to increase their potential for digestion and absorption.

There is a dearth of research reports on the effects of dietary factors, especially those evaluated in the current study on intestinal morphology and digestive function. Our results indicate some negative effect of feed restriction on the development of the crypt in the jejunum. The crypt is the region associated with the renewal of the mucosa, both structurally and functionally [16, 34, 36]. The effect of feed restriction on the crypt did not, however, result in shorter villi, except at the lowest level of dietary protein. The absence of any negative effect on the villus may be due to the fact that villus growth is regulated by both cell formation in the crypt and the rate of migration and extrusion. It is not known how feed restriction or quality affects the latter processes (migration and extrusion). The absence of negative effects of the UPS on mucosal protein content suggests that tissue protein synthesis or loss was not affected by the treatments. This result is, however, contradicted by the negative effect of feed restriction on the mucosal protein content in the jejunum.

The effects of the dietary treatments on digestive enzyme activities appear to be dependent on the nature of the enzyme. The key enzymes assessed in the present study, maltase and sucrase are carbohydrases and were chosen because of the relatively high levels of carbohydrates in the poultry diets. The activities of sucrase and AP were negatively affected by dietary protein content but only on diets of low protein quality. This finding is in contrast to research reports on the rat [35]. In the rat, the activity of an aminopeptidase, a protease, was low in low protein diets while there were no effects of protein level on the activities of sucrase and AP.

There are numerous reports on the effects of protein level, protein quality and feed restrictions on the growth of broiler chickens [11, 36, 41]. The effects of feed restriction on the gross development and mucosal morphometry of chicks has also been examined by previous researchers [1, 2]. The present research is probably the first to comprehensively examine the effects of feed quality, protein level and feed restriction on the development of the GIT in broiler chickens. In response to protein quality, the changes in feed intake, body growth and FCE were in the same direction as those of mucosal morphometry and digestive enzyme activities. Crude protein content and feeding level did not produce the same clear-cut relationships between gross response and mucosal structure or digestive function. The effect of feed restriction in early life may be confounded by the preferential utilisation of nutrients for intestinal development, as was previously highlighted [17, 31]. The lack of clear-cut relationships between protein content, digestive function and animal response may also be due to the nature of the enzymes that were assessed in the current study.

5. CONCLUSION

The overall superior growth performance of birds fed the BPS confirmed the principle that diets which supply an array of nutrients, i.e. energy or protein that closely meet the broiler's nutrient needs give rise to a better performance. The poorer performance observed for birds fed excess protein with low E:P ratios (i.e. 32.5 MJ ME·kg^{-1} protein) suggests that when the dietary protein content is increased beyond that required to meet the amino acid requirements of a broiler, the energy content should also

be increased to ensure that sufficient energy is available for the efficient utilisation of dietary protein. The effects of feed quality and protein content provide an insight into the regulation of intestinal growth by feed factors. The changes in the activities of some of the digestive enzymes explain the effects of protein quality and daily food supply on body growth. Some link has also been established between feed factors, gastrointestinal physiology and animal response. This is an area that warrants further investigation.

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