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Body Growth, Visceral Organ Weight and Intestinal Digestive Enzyme Fchickens on Diets Varying in Energy and Protein Contents

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Abstract: The effects of varying dietary energy and protein (E:P) ratios on the development of the gastrointestinal tract and biological performance of broiler chickens (10-24d) were evaluated. Changes in dietary protein level significantly ($P<0.001$) influenced feed intake, body weight gain and feed conversion efficiency, this being most profound at dietary energy levels of 11, 12 and 13 MJ/kg. Body eight was reduced ($P<0.001$) as dietary E:P ratios decreased at constant dietary energy level. The weights of some visceral organs were also affected by dietary treatment. These included a reduction ($P<0.05$) in the weight of the proventriculus/gizzard with an increase in dietary protein and energy contents. At a dietary energy level of 12 MJ/kg, the weight of the pancreas rose ($P<0.001$) with an increase in dietary protein content. The jejunal protein content was affected ($P<0.01$) by dietary protein level and interactions between dietary energy and protein. Mucosal protein was lowest at the highest dietary E:P ratios within the 11, 12 and 13 ME series. Maltase activity in the jejunum was influenced ($P<0.05$) by dietary energy, being lowest ($P<0.05$) in chicks that were fed diets containing 14 MJ ME/kg. An increase in dietary E:P ratio resulted in an increase in the activity of sucrase ($P<0.001$) and AP ($P<0.05$) for birds fed diets in the 11, 12 and 13 ME series. Overall, our findings suggest that the differences in biological performance of chicks fed diets varying in energy and protein contents may be traceable to a lack of energy for metabolic function. The higher the amount of mucosal protein the greater may be the bird's digestive function and absorptive capacity. Some of the effects of varying dietary E:P ratio also appear to be linked to changes in intestinal digestive function.

Key words: Energy to protein ratio, broiler chicken, intestinal growth and enzyme activity

Introduction

The effects of varying dietary energy and protein contents on intestinal function are less well understood than the growth response of birds. It is generally assumed that the changes in productivity observed are moderated by nutrient metabolism at the internal body tissues and alteration to the processing of energy and nutrients, including protein. Nutrient processing by the gastrointestinal tract (GIT) determines the amount of nutrient that is available to the internal tissues for metabolism. The GIT also utilizes an enormous amount of nutrients for self-renewal (Webster, 1980; Reynolds *et al*., 1991). In addition, the efficiency of nutrient supply to internal tissues would be dependent on dietary factors, including dietary energy and protein contents.

The GIT of the chicken is anatomically complete in the embryonic phase (Moran, 1985). However, it develops functionally to a peak within a few days after hatching (Nitsan *et al*, 1991; Iji *et al*., 2001a,b). Sulistiyanto *et al*. (1999) indicated that the intestinal villi, which are known to play a crucial role in digestion and absorption, are underdeveloped at hatching. The growth of villi is partly genetically dependent, and is stimulated by the presence of external factors, including dietary nutrients, to attain maximum capacity at about 10 d after hatching (Moran, 1985; Noy and Sklan, 1995). Noy and Sklan (1995) suggested that nutrient availability in chicks developed concomitantly with the growth of digestive organs and the increase in enzyme activities. To ensure maximum growth thereof, the utilization of balanced amounts of nutrient:energy ratios are a prerequisite to allow the GIT to reach optimum capacity during the early growth period (viz: 1-21 d) in the chick. The objective of this study was to examine the capacity of the broiler chick to utilize diets varying in energy and protein content and to determine the response of the GIT to dietary treatments.

Materials and Methods

Four hundred and eighty Ross commercial male

Table 1: The raw ingredient composition and nutrient contents of the basal feeds blended to produce experimental diets.

Raw ingredient (g/kg)	Basal diet 1	Basal diet 2	Basal diet 3	Basal diet 4
Maize	584.9	27.0	57.9.9	99.9
Sugar	0.0	89.9	0.0	0.0
Maize gluten 60	79.9	119.9	55.9	99.9
Sunflower O/C 37	48.6	1.5	138.8	158.4
Soybean 50	27.0	98.7	75.4	118.1
Soya protein isolate 66	67.3	454.0	0.0	352.1
Fishmeal 65	106.0	109.9	104.5	108.6
Vegetable oil	62.8	81.3	0.0	45.3
Limestone	10.1	7.7	9.4	6.8
Sodium bicarbonate	1.1	0.6	1.2	0.7
MCP[1]	8.7	6.3	9.2	7.0
Vitamins + Minerals	2.5	2.5	2.5	2.5
Sodium bicarbonate	1.1	0.6	1.2	0.7
Filler	0.0	0.0	22.0	0.0
Nutrient composition (g/kg):				
Dry matter	895.7	917.7	879.3	919.7
Crude protein	250	500.0	250.0	500.0
AMEn (MJ/kg)	14.0	14.0	11.0	11.0
Fat 100.0	100.0	38.0	68.0	
Calcium 10.0	10.0	10.0	10.0	
Crude fibre	23.1	24.2	33.7	43.0
Phosphorus (available)	5.0	5.0	5.0	5.0
Sodium	1.5	1.5	1.5	1.5
Chloride	1.8	1.8	1.8	1.8

[1]MCP Monocalcium phosphate

broiler chicks were housed, 10 birds to a cage, in single-tiered, wire-floored brooder cages. Each cage was equipped with its own feeder and two nipple drinkers. Food and water were provided *ad libitum*. Four basal diets (Table 1) were formulated to contain either 250 or 500 g crude protein/kg and 11.0 or 14.0 MJ AME/kg. Appropriate blending produced 12 dietary mixtures varying in E:P ratio. There were thus four energy levels (11, 12, 13 and 14 MJ AME/kg) and three protein levels (250, 400, 500 g/kg). The experimental design was therefore, a 4 x 3 factorial design, yielding 12 E:P ratios varying from 22 to 56 MJ AME/kg protein. Birds (initial body weight 208±14.4g) were randomly allocated to the multi-bird cages. There were four cage replicates per treatment. The chicks were fed one of the 12 diets between 10 and 24 days of age. The food allocated to each pen of birds at the start of each week and that remaining at the end of the week was recorded, as were the weights of the chicks. At the end of the 14-d experimental period, three birds per cage were slaughtered through asphyxiation with carbon dioxide. The birds were dissected and the GIT removed. The full and empty weights of different regions of the tract were measured, to obtain both the tissue weight and the digesta holding capacity (DHC). The proventriculus plus gizzard, duodenum, jejunum, ileum and caeca as well as pancreas and liver were weighed.

Analysis of Digestive Enzyme Activities: Brush-border membrane vesicles (BBMV) were prepared in line with the method described by Shirazi-Beechey *et al.* (1991). The preparation of BBMV entails hypotonic shock and rupture of the cell, followed by serial centrifugation and elimination of unwanted cell fractions. The end-product is devoid of contamination from the pancreas or digesta. The specific activities of brush-border membrane-bound carbohydrases, maltase (EC. 3.2.1.20) and sucrase (EC. 3.2.1.26) were measured, using a modification of the method described by Dahlqvist (1964). Incubation released glucose which was then estimated by the GOD-Perid test kit (glucose oxidase, EC. 1.1.3.4) (Roche Diagnostics, Indianapolis, USA). The amount of glucose released was determined colorimetrically at 610 nm after 30 minutes of colour development at room temperature. The

activities of total protease and alkaline phosphatase (AP, EC. 3.1.3.1) were also measured according to techniques used by Holdsworth (1970) and Kopecny and Wallace (1982).

Data Analysis: Data were subjected to ANOVA, using the General Linear Model and multiple regression to examine the response of the variables to varying rates of the tested factors (Minitab Inc., 1998).

Results

The response of the birds, in terms of feed consumption and utilization is shown in Table 2. The interactions between the energy and protein factors were not significant and therefore responses to protein were independent of energy. Variation in dietary protein level had significant ($P<0.001$) effects on feed intake, final body weight, weight gain and feed conversion efficiency (FCE). The effects of varying protein on feed intake and FCE were most profound at dietary energy levels of 11, 12 and 13 MJ/kg, these tending to decline as dietary E:P ratios decreased. Similarly, final body weight declined ($P<0.001$) with a reduction in E:P ratios (i.e. beyond 250 g CP/kg) but this occurred only at dietary energy levels of 12 and 13 MJ/kg. The effects of increasing protein content (i.e. decreasing E:P ratios) over 250 g CP/kg on body weight gain were observed at all energy levels assessed. Body weight gain also responded ($P<0.05$) positively to increasing dietary energy level between 11 and 13 MJ/kg. FCE generally increased ($P<0.001$) with increase in dietary energy content.

The weight of the proventriculus and gizzard declined ($P<0.05$) with a decrease in E:P ratios below the range 44 to 56 MJ ME/kg, especially at the higher dietary energy levels, 13 and 14 MJ/kg (Table 3). The weight of the proventriculus and gizzard was also influenced ($P<0.05$) by dietary energy level, tending to decline with an increase in dietary energy. Duodenal weight, however, increased ($P<0.01$) with decreasing E:P ratios but this was only noticeable in birds fed diets in the 11 MJ/kg series. There was a significant ($P<0.01$) interaction between dietary protein and energy on the weight of the duodenum. The weights of the jejunum, ileum and caeca were not affected by variation in dietary energy or protein. At a dietary energy level of 12 MJ/kg, the weight of the pancreas increased ($P<0.001$) as dietary E:P ratios decreased. The weight of the pancreas increased ($P<0.05$) with increasing dietary energy between 11 and 12 MJ/kg although this was noticeable only at E:P ratios of 22 and 24 MJ ME/kg respectively.

There was a slight increase in mucosal protein content with an increase in ME series from 11 to 12 MJ ME/kg and a decline thereafter. The mucosal protein content of the jejunum was affected ($P<0.01$) by the interactions between dietary energy and protein (Table 5). At a dietary energy content of 11 MJ/kg, jejunal protein tended to rise with decreases in E:P ratios while the reverse was the case at a dietary energy level of 14 MJ/kg. The activity of maltase, sucrase and AP (not protease) increased slightly for birds fed diets in the 11 to 12 ME series and thereafter showed a declining trend with the lowest activity being recorded on the 14 ME series. The interaction between energy and protein significantly ($P<0.05$) influenced the activity of jejunal maltase. Maltase activity was lowest ($P<0.05$) in chicks on the 14 MJ/kg diets, regardless of the dietary E:P ratios or crude protein content. There was no impact of dietary energy on the activity of sucrase at the lowest dietary E:P ratios within each ME series. However, sucrase activity was influenced ($P<0.01$) by variation in dietary energy at other protein levels. Similarly, sucrase activity varied ($P<0.001$) with dietary E:P ratios for birds fed diets between the 11 and 13 ME series, and the effects of energy x protein were significant ($P<0.001$). The activity of AP increased with increasing E:P ratios within each ME series. There was also a significant ($P<0.05$) interaction between dietary protein and energy on the activity of AP in the jejunum. In general, mucosal protein, the activity of maltase, sucrase and AP declined, but this was dependent on the dietary protein content.

Discussion

Relatively large biological responses were obtained with diets with E:P ratios of between 52 to 56 MJ AME/kg (13 to 14 MJ/kg, 250 to 300 g/kg CP). At present, it is recommended that a diet with 250 g/kg protein and 13 MJ/kg be provided for a broiler in the starter period. The ME:CP ratio of this diet is 52 MJ AME/kg, which suggests that broilers in the starter period are being fed close to their optimum for growth. However, as genetic selection for growth rate (leanness) continues, so does the protein requirement of the bird increase (Faulkner, 1993). It is mandatory, therefore, that if the protein content of the feed is increased, the energy content should, likewise, be increased so as to maintain this E:P ratio. The poor performance of birds fed diets at the lowest E:P ratios may be due to shortfall in energy required for protein metabolism.

The small intestine of birds exhibits considerable

Table 2: Effects of varying dietary energy:protein ratios on the biological performance of broiler chicks.

Energy (MJ ME/kg)	Protein (g/kg)	E:P ratio (MJ ME/ kg protein)	Initial weight (g)	Final weight (g)	Weight gain (g/day)	Feed intake (g/day)	FCE (g wt gain/kg feed)
11	500	22.0	215.8	506.1^{c}	20.7^{d}	45.4^{c}	460^{c}
	400	27.5	205.7	562.9^{bc}	26.7^{cd}	49.9^{bc}	530^{bc}
	250	44.0	199.9	680.1^{abc}	36.1a^{bc}	61.8^{ab}	580^{ab}
12	500	24.0	213.9	535.3^{c}	23.0^{d}	46.2^{c}	500^{c}
	400	30.0	202.2	583.3^{bc}	28.6^{cd}	53.0^{bc}	540^{bc}
	250	48.0	223.4	727.3^{ab}	38.0^{abc}	64.7^{ab}	590^{ab}
13	500	26.0	225.2	571.3^{c}	26.0^{d}	48.3^{c}	540^{bc}
	400	32.5	196.7	619.6^{bc}	32.1^{cd}	50.0^{c}	640^{a}
	250	52.0	189.6	741.9^{a}	43.8^{a}	65.3^{ab}	670^{a}
14	500	28.0	208.1	555.3^{bc}	26.3^{d}	43.2^{c}	610^{ab}
	400	35.0	222.4	704.6^{ab}	36.3^{bc}	57.8^{bc}	630^{ab}
	250	56.0	189.2	684.6^{ab}	37.3^{bc}	54.5^{bc}	680^{a}
		SEM	14.14	50.32	3.70	4.44	34.0
Source of variation	**Energy level**		NS	NS	*	NS	***
Protein level			NS	***	***	***	***
Energy x protein			NS	NS	NS	NS	NS

a,b,c - Mean values in the same column not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001). SEM - standard error of difference between mean values.

Table 3: Effects of varying dietary energy:protein ratios on empty weights of visceral organs (g/100 g body weight) of chicks.

Energy (MJ/kg)	Protein (g/kg)	E:P ratio (MJ/kg protein)	Gizzard[1]	Duodenum	Jejunum	Ileum	Caeca	Pancreas	Liver
11	500	22.0	3.5^{ab}	1.1^{a}	1.5	1.3	0.7	0.4^{bc}	4.5
	400	27.5	4.0^{a}	0.9^{ab}	1.2	1.0	0.7	0.5^{ab}	4.1
	250	44.0	3.7^{a}	0.8^{b}	1.2	1.0	0.5	0.4^{bc}	3.9
12	500	24.0	3.7^{a}	1.0^{ab}	1.4	1.3	0.7	0.6^{a}	4.2
	400	30.0	3.8^{a}	0.8^{b}	1.2	0.9	0.6	0.5^{ab}	4.1
	250	48.0	3.9^{a}	1.0^{ab}	1.3	0.9	0.5	0.4^{bc}	3.9
13	500	26.0	3.2^{b}	0.8^{b}	1.1	1.0	0.5	0.4^{bc}	3.9
	400	32.5	3.1^{b}	0.9^{ab}	1.2	1.1	0.6	0.4^{bc}	3.8
	250	52.0	3.7^{a}	0.9^{ab}	1.3	1.1	0.6	0.3^{c}	4.1
14	500	28.0	3.2^{b}	1.0^{ab}	1.6	1.2	0.7	0.4^{bc}	4.4
	400	35.0	3.8^{a}	0.8^{b}	1.3	1.1	0.6	0.4^{bc}	4.1
	250	56.0	3.8^{a}	0.8^{b}	1.3	1.0	0.5	0.4^{bc}	3.8
	SEM	0.47	0.09	0.16	0.13	0.20	0.12	0.68	
Source of variation									
Energy level			*	NS	NS	NS	NS	*	NS
Protein level			*	**	NS	NS	Ns	***	*
Energy x protein			NS	**	NS	NS	NS	NS	NS

a,b,c - Mean values in the same column not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001). SEM - standard error of difference between mean values.

1. Including the proventriculus.

variation in length and probably weight (per unit body weight), depending on the type of feed the bird eats (Low and Zebrowska, 1986). Results of the present experiment show that dietary energy and protein contents did exhibit a clear effect on GIT weights. Large amounts of nutrients are used at the intestinal level for self-sustenance and renewal. These requirements have not been established for poultry but in cattle, sheep and pigs, over 20 % of the whole-body consumption of oxygen occurs at the level of the GIT (Webster, 1980; Reynolds *et al.*,

Table 4: Effects of varying energy:protein ratios on digesta holding capacity (g/100 g body weight) of the gastrointestinal tract.

Energy (MJ/kg)	Protein (g/kg)	E:P ratio (MJ/kg)	Proventriculus+Gizzard	Small intestine	Caeca
11	500	22.0	1.0^{ab}	2.8^{b}	0.3^{cd}
	400	27.5	1.3^{a}	3.0^{ab}	0.3^{cd}
	250	44.0	1.3^{a}	3.4^{ab}	0.2^{d}
12	500	24.0	1.1^{ab}	2.9^{ab}	0.4^{bcd}
	400	30.0	1.2^{a}	2.8^{bc}	0.4^{bcd}
	250	48.0	1.0^{ab}	3.9^{a}	0.3^{cd}
13	500	26.0	0.8^{bc}	2.6^{b}	0.4^{bcd}
	400	32.5	0.8^{bc}	2.2^{bc}	0.5^{ab}
	250	52.0	1.0^{ab}	2.8^{bc}	0.6^{a}
14	500	28.0	0.6^{c}	1.6^{c}	0.5^{ab}
	400	35.0	0.8^{bc}	2.8^{bc}	0.5^{ab}
	250	56.0	1.2^{ab}	2.8^{bc}	0.4^{bcd}
		SEM	0.15	0.38	0.06
Source of variation					
Energy level			***	***	***
Protein level			**	***	NS
Energy x protein			NS	NS	**

a,b,c - Mean values in the same column not sharing a superscript are significantly different (**P<0.01; ***P<0.001). SEM - standard error of difference between mean values.

Table 5: Effects of varying energy:protein ratios on mucosal protein, brush-border membrane protein (mg/g tissue) and specific activities of membrane-bound enzymes (ìmole product/mg protein/minute) in the jejunum.

Energy (MJ/kg)	Protein (g/kg)	E:P ratio (MJ/kg)	Mucosal protein	Membrane protein	Maltase	Sucrase	Protease (Units)	AP
11	500	22.0	63.9^{ab}	1.03^{a}	10.2^{bc}	0.44^{c}	9.9	9.5^{bc}
	400	27.5	69.1^{a}	1.10^{a}	14.6^{a}	0.63^{abc}	10.0	9.8^{ab}
	250	44.0	47.9^{c}	0.79^{abc}	10.5^{bc}	0.65^{ab}	0.0	7.2^{bc}
12	500	24.0	63.3^{ab}	0.99^{a}	11.1^{bc}	0.48^{bc}	9.7	7.2^{bc}
	400	30.0	66.5^{ab}	0.74^{abc}	14.8^{a}	0.75^{a}	9.9	15.1^{a}
	250	48.0	54.4^{b}	0.81^{abc}	12.6^{abc}	0.69^{ab}	10.7	12.1^{ab}
13	500	26.0	55.0^{bc}	0.55^{c}	13.5^{ab}	0.67^{ab}	10.0	8.5^{bc}
	400	32.5	73.7^{a}	0.78^{abc}	10.6^{bc}	0.43^{c}	10.0	8.1^{bc}
	250	52.0	51.8^{bc}	0.65^{bc}	11.6^{abc}	0.78^{a}	10.1	10.0^{abc}
14	500	28.0	51.2^{c}	0.97^{a}	9.6^{c}	0.43^{c}	9.9	6.3^{c}
	400	35.0	58.0^{abc}	0.88^{abc}	9.7^{c}	0.48^{bc}	9.4	6.2^{e}
	250	56.0	60.9^{abc}	0.88^{abc}	9.9^{c}	0.54^{bc}	9.3	7.4^{bc}
		SEM	5.18	0.151	1.62	0.078	0.42	1.98
Source of variation								
Energy level			NS	**	*	**	NS	**
Protein level			**	NS	NS	***	NS	NS
Energy x protein			**	NS	*	***	NS	*

a,b,c - Mean values in the same column not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001). SEM - standard error of difference between mean values.

1991). Most of the energy utilized by the GIT is channelled towards protein synthesis. In recent studies, Iji *et al.* (2001a) observed large changes in villus height and mucosal cell turnover in broiler chickens between hatch and 21 days of age. These changes may be supported by large amounts of nutrients, including protein.

It is not certain why there was a reduction in the weight of pancreas with increase in dietary energy. The pancreas is a major source of digestive enzymes although pancreatic enzymes were not assessed in the current study. The intestinal

enzymes that were tested generally responded to variation in dietary E:P ratios.Normal development of digestive function is stimulated by the introduction of feed (Baranyiova and Holman, 1976; Moran, 1985) and enzyme activities generally respond to the presence of target substrates. The pattern of development in broiler chickens, in response to variation in dietary energy to protein contents (i.e. E:P ratios) has not been reported. There have been more extensive studies on the effects of age on intestinal enzyme activities in other strains of broiler chickens (Uni *et al.*, 1995a,b; Iji *et al.*, 2001b). Takahashi and Akiba (1996) reported on the effects of varying dietary protein on the activities of hepatic malic enzyme and fatty acid synthetase in male broiler chicks. The activities of these enzymes were higher in chicks on a low protein diet than in chicks on high protein. The responses of digestive enzymes in the GIT may be more directly related to the concentrations of their substrates and may differ from those of the liver, which are mostly involved in synthesis or detoxification. The findings are in agreement with the results of Iji *et al.* (2001b) in which the specific activity of maltase was found to be higher than that of other enzymes.

It could be speculated that the overall poor performance of birds on some diets could be partly ascribed to an underdeveloped GIT. A decrease in food intake by birds exhibiting poor performance may be due to a lack of intake of adequate amounts of nutrients, to meet the needs for cell renewal and growth of the GIT and the other cells of the body. Dietary E:P ratios within the range 22 to 32 MJ ME/kg stimulated the deposition of protein in the intestinal mucosa, while higher E:P ratios within the range of 44 to 52 MJ ME/kg resulted in a decline in the concentration of mucosal protein. It must be noted that intestinal mucosal protein content is a rough estimate of cell size (Waterlow *et al.*, 1978). Therefore, the higher the amount of mucosal protein the greater may be the bird's digestive ability and absorptive capacity, although most of the mucosal protein may be present as structural, rather than functional protein. The exact effects of the two factors on digestive enzymes may be a better indication of their effects on digestion. In the present study, the major changes in digestive enzyme activities were due to interaction between energy and protein. This effect may be due to the fact that both maltase and sucrase are involved in carbohydrate digestion (Iji *et al.*, 2001b). The exact role of AP is unknown but there are suggestions that it may be involved in nutrient transport and this function could be regulated by dietary energy to protein supply.

Conclusions

The effects of dietary energy and protein at varying E:P ratios on the development of the gastrointestinal tract and biological performance of broiler chickens (10-24d) were evaluated. Changes in dietary protein level significantly ($P<0.001$) influenced feed intake, body weight gain and feed conversion efficiency. Performance decreased ($P<0.001$) as dietary E:P ratios decreased (i.e. 500 g CP/kg). The weights of some visceral organs were also affected by dietary treatment. These included a reduction ($P<0.05$) in the weight of the proventriculus and gizzard with an increase in dietary protein and energy contents. The jejunal protein content was affected ($P<0.01$) by dietary protein level but the response was dependent on dietary energy. Mucosal protein was lowest at the highest dietary E:P ratios within the 11, 12 and 13 ME series. Maltase activity in the jejunum was influenced ($P<0.05$) by both dietary energy and protein, being lowest ($P<0.05$) in chicks that were fed diets containing 14 MJ ME/kg and 500 g CP/kg. An increase in dietary E:P ratio resulted in an increase in the activity of sucrase and AP for birds fed diets in the 11, 12 and 13 ME series. Overall, these findings suggest that the differences in biological performance of chicks fed diets varying in energy and protein contents may be traceable to a lack of energy for metabolic functions. The higher the amount of mucosal protein the greater may be the bird's digestive function and absorptive capacity. There may also be changes in intestinal growth and function but these would require further evaluations.

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