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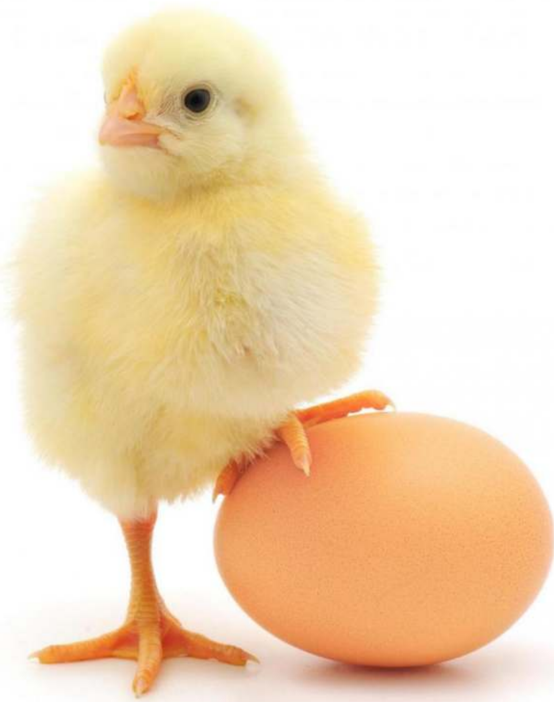

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Effects of Different Protein and Energy Contents of the Diet on Growth Performance and Hormonal Parameters in Two Commercial Broiler Strains

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Abstract: The present study was carried out to investigate the influence of energy and protein contents of the diet on growth performance and to evaluate further interactions of dietary manipulation and genotype on endocrine parameters in broiler chickens. 1200 day-old male and female broiler chicks from 2 commercial broiler strains (Hybro and Ross) were fed *ad libitum* from day 1 onwards by 4 different diets either with a high or low energy combined with a high or low crude protein content. Body weight and feed intake were measured weekly. Venous blood samples were taken (10 birds/sex /line/diet group) weekly from day 14 on, for plasma Growth Hormone (GH), Triiodotyronine (T_3) and Thyroxin (T_4) content. Relative growth rate was higher in broiler chickens which fed the low energy diet compared to high energy diet during the first week of age, while from week 1 on, a reverse effect of energy on relative growth rate was found. The effect of energy and protein on feed intake was significant ($p<0.05$), while no interaction between energy and protein was found. Within high energy diet, birds fed low protein diet showed a tendency for higher T_3 levels, while on low energy diet, broiler chicks fed high protein level showed a tendency for higher plasma T_3 concentrations, probably explaining the presence of a significant energy×protein interaction. Protein content of the diet, significantly ($p<0.05$) affected plasma T_4 levels while energy content had no effect. The higher GH as well as T_3 levels in the younger chicks may be an indicator of higher requirement of these hormones for protein synthesis during the fast growth phase. It can be concluded that higher protein content of the feed could result in lower T_3, higher T_4 and higher plasma GH levels. Possible causal mechanisms underlying this phenomenon are discussed.

Key words: Diet, energy, protein, broilers, GH, T_3, T_4

Introduction

It is generally accepted that (neuro) endocrine factors act as an interface between input factors such as genotype, sex, environmental conditions, nutrition, etc. on one hand and the realized outputs such as growth rate, feed efficiency, carcass composition, etc. on the other hand, by which these factors affect metabolism processes (Buyse *et al.,* 2001). As it is for mammalian species, GH is indispensable for normal growth and development of avian species. But in contrast of mammals, exogenous GH administration has little, if any, potential for improving the growth rate and feed efficiency of rapidly growing broilers; it is more likely to do so in older birds. This is at least partly because of age-related changes in tissue GH-binding activity and GH-receptor mRNA expression (Buyse and Decuypere, 1999). The realized body weight gain and its compositional changes are the result of complex interactions between endogenous and exogenous factors (Decuypere *et al.,* 1991). From the study of Scanes *et al.* (1981), apparently a reduction in dietary protein content was associated with higher plasma concentrations of GH, which plays an important role in avian growth. Furthermore, Buyse *et al.* (1992) presented evidence that the pattern of growth hormone secretion was markedly affected by dietary treatment. Broilers fed low protein diet had higher overall mean, amplitude, baseline and peak frequency of plasma GH than broiler chickens fed high protein diet. It was found that broiler chicks reared on the low protein diet produced more heat per kg of metabolic body weight ($kgW^{0.75}$) than broiler chicks fed on the high protein diet. Recently, it was shown that genes of the somatotropic axis respond to nutrition differently at the level of gene transcription (Zaho *et al.,* 2004).

The thyroid gland and its secretions appear to play a role in the endocrine regulation of growth in humans and other higher primates, as they do in other mammals and in birds (Darras *et al.,* 1994). It was found that mean daily circulating T_3 levels rise rapidly in the first 2 weeks after hatching, then decline with age, resulting in a good correlation between circulating T_3 levels and relative growth (Kühn *et al.,* 1982; Decuypere, 1988). In addition, feeding pattern plays an important role in the regulation of the daily rhythm in the concentration of plasma thyroid hormones (Klandorf *et al.,* 1981; Decuypere and Kühn, 1984). Following fasting the rhythmicity of T_3 but not T_4 disappears (Klandorf *et al.,* 1981; Decuypere and Kühn, 1984), indicating that the diurnal variation on the T_3 to T_4 conversion, related to the pattern of feed intake is important in determining the T_3-rhythm (Decuypere and

Kühn, 1984; Sharp and Klandorf, 1985). The dietary protein restriction was associated with higher circulating plasma T_3 and lower plasma T_4 levels (Kühn *et al.,* 1990; Buyse *et al.,* 1992). In other studies chickens were fed the low protein diet was characterized by the lowest plasma T_3 and T_4 (Vasilatos Younken *et al.,* 1999; Malheiros *et al.,* 2003). Further studies on growing chickens suggested that GH might be important in regulating plasma T_3, since injection of GH-antiserum provoked a decrease in circulating T_3 levels (Darras *et al.,* 1993). This hypothesis was confirmed by experiments on chickens taken 4-7 days after hypophysectomy. In these animals endogenous GH is almost undetectable while the amount of hepatic GH binding sites is increased (Vanderpooten *et al.,* 1991). Plasma T_4 and hepatic outer ring deiodinase-I levels are only slightly affected, but plasma T_3 is profoundly decreased, while hepatic inter ring deiodinase-III activity increased (Darras *et al.,* 1993). GH injection in this hypophysectomized chickens again increases plasma T_3, together with a decrease in both hepatic inter ring deiodinase-III and GH receptor number (Darras *et al.,* 1994). One way of getting more insight in the hormonal regulation of growth is measuring the functional activity of involved hormonal axes during growth phase. The present study was designed to examine the influence of different energy and protein contents of diet on growth performance and to test further interactions of dietary manipulation and genotype on endocrine parameters in broiler chickens.

Materials and Methods

Animals and Management: A total of 1200 day-old male and female broiler chicks from 2 commercial lines (Hybro and Ross) were allotted at randomly in equal numbers in floor pens. Wood shavings were used as litter and a nearly continuous lighting regimen including 23h lightness and 1h darkness throughout the rearing period were provided. Chicks were fed with 4 different diets, high energy diet (3300 Kcal ME/Kg) with high (24%) (D1) or low (20%) (D2) Crude Protein content (CP), or low (20%) (D4) CP content. Water and feed were available *ad libitum*. Temperature was set at 30°C during the first week and gradually reduced by 2°C per week down to 20°C. The experiment was conducted in a completely randomized design by a factorial arrangement of treatments.

Experimental procedure: From day 7 onwards, and repeated weekly, chickens were weighed on a pen basis and weekly feed intake per pen determined. Blood sample was collected (10 birds/sex/line/diet group) weekly by using a heparinized syringe. Blood samples were centrifuged and plasma stored frozen until assayed for GH, T_3 and T_4. Plasma GH concentrations were measured by a homologous radioimmunoassay. The intra- and inter-assay coefficients of variation were 4% and 15.5% respectively and no cross reactivity with other chicken pituitary hormones was observed. The characterization of the cGH and the specificity of the monoclonal antibodies (murine monoclonal anti-cGH) used to develop the assay have been discussed by Berghman *et al.* (1988). Measurements of the T_3 concentrations in the plasma were performed by radioimmunoassay using a commercially available T_3 antiserum, rabbit anti-T_3 in combination with a specific tracer $[^{125}I]T_3$. The cross reactivity with T_4 was 0.1-0.5% and intra-assay and inter-assay coefficients of variation were 2.9% and 6.2% respectively. The T_4 concentrations in plasma were assayed using specific tracer $[^{125}I]T_4$ and a laboratory-raised rabbit anti-T_4 antiserum (Huybrechts *et al.,* 1989). This T_4 antiserum had a 0.16% cross reactivity with T_3, an intra-assay coefficient of variation 3.2% and an inter-assay coefficient of variation of 10.1%.

Statistical analysis: Values are expressed as means±SEM. Growth performance results for live weight, feed intake, feed conversion ratio and triiodothyronine, thyroxin and growth hormone data were analyzed by means of analysis of variance based on factorial arrangement of treatments with feed, line, sex and age as classification variables and interactions between them were calculated. In addition, if the overall model was significant (F test, $p<0.05$), treatment means were compared with Duncan's multiple range test (SAS, 1988).

Table 1: Results of the analysis of variance (P values) according to classification variables: age, diet, breed, sex and their interaction for RG, FI, FCR, plasma T_3, T_4 and GH levels

Variables	RG	FI	FCR	T_3	T_4	GH
Age (A)	P<0.001	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Diet (D)	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05
Breed (B)	P<0.05	P<0.01	P<0.05	NS	NS	NS
Sex (S)	P<0.05	P<0.01	P<0.01	NS	P<0.02	P<0.01
A × D	P<0.001	NS	P<0.02	P<0.01	NS	P<0.01
A × B	P<0.01	P<0.01	P<0.01	NS	NS	P<0.01
A × S	P<0.01	P<0.01	P<0.01	NS	P<0.01	NS
B × D	NS	NS	NS	NS	P<0.05	NS
B × S	NS	NS	NS	NS	NS	NS
D × S	NS	NS	NS	NS	NS	NS

RG = relative growth, FI = feed intake, FCR = feed conversion ratio

Table 2: Weekly relative growth rate (%) in commercial broilers of two different strains (H: Hybro and R: Ross) and two sexes (F: female and M: male). Values are mean±SEM

	Strain		Sex	
Week	H	R	F	M
1	254±4.9[a]	258±5.5[a]	252±4.9[a]	259±5.4[a]
2	152±2.9[b]	154±3.7[b]	149±3.7[b]	158±2.9[b]
3	78±1.9[c]	86±1.8[c]*	80±1.1[c]	84±2.5[c]
4	67±2.4[d]	67±1.4[d]	65±1.1[d]	69±2.6[d]
5	41±1.0[e]	40±1.0[e]	39±0.8[e]	42±1.2[e]
6	35±1.1[e]	35±1.2[e]	31±0.8[e]	39±1.1[e]*

a, b, c, d, e, means within columns having different superscripts are significantly different (p<0.05). Values followed by asterisk indicate significant differences between the two types of strain and sex at each sampling time (p<0.05)

Table 3: Weekly body weight (g/chicken) in commercial broilers fed four different diets. Values are mean±SEM

	Diets			
Week	D1	D2	D3	D4
1	136±2[b]	141±3[ab]	145±3[a]	147±3[a]
2	364±5	376±6	380±6	362±9
3	665±24[b]	713±9[a]	693±11[ab]	659±12[b]
4	1140±16[b]	1183±13[a]	1133±14[b]	1134±13[b]
5	1647±29[ab]	1706±21[a]	1625±27[b]	1623± 26[b]
6	2217±59	2297±48	2142±51	2185±45

a, b, means within rows having different superscripts are significantly different (p<0.05)

Results

Growth performance measurements: Results of the analysis of variance are presented in Table 1. Evidently, Relative Growth (RG) were highly dependent on the age (p<0.001) of the birds. Additionally, strain and sex significantly affected relative growth (p<0.05). Ross birds tended to show faster growth than Hybro until week 3 of age and this was statistically significant (p<0.01) at third week. Male birds tended to show a higher relative growth than females and this was only significant (p<0.01) at week 6 (Table 2). Diet had no significant effect on the relative growth rate, but significant interactions (p<0.001) between diet×age were found (Table 1). The absolute body weight in birds fed D3 and D4 (low-energy diets) was superior to that of their D1 and D2 (high-energy diets) fed counterparts during the first week of age, while from week 1 on, a reverse effect of energy on body weight was found (Table 3).

Feed consumption: Strain, age and diet had significant effect on the feed intake (Table 1). Hybro birds ate more than Ross and male chickens in both breeds consumed more feed than female birds (Table 4). Broilers fed the D2 diet had a significantly lower feed intake than the other groups at 2, 4, 5 and 6 weeks of age (Table 5). The effect of energy and protein levels on feed intake was significant (p<0.01), while no interaction between

Table 4: Weekly feed intake (g/chicken) in commercial broilers of two different strains (H: Hybro and R: Ross) and two sexes (F: female and M: male). Values are mean±SEM

	Strain		Sex	
Week	H	R	F	M
1	161±2[f]	145±3[f]*	153±3[f]	153±3[f]
2	328±4[e]	305±6[e]*	307±3[e]	327±5[e]*
3	528±13[d]	495±9[d]*	495±10[d]	526±12[d]*
4	761±19[c]	737±189[c]	706±19[c]	795±15[c]*
5	876±31[b]	834±27[b]	805±27[b]	906±30[b]*
6	1092±39[a]	943±36[a]*	972±27[a]	072±48[a]*

a, b, c, d, e, f, means within columns having different superscripts are significantly different (p<0.05). Values followed by asterisk indicate significant differences between the two types of strain and sex at each sampling time (p<0.01)

Table 5: Weekly feed intake (g/chicken) in commercial broilers fed four different diets. Values are mean±SEM

	Diets			
Week	D1	D2	D3	D4
1	162±6[ab]	146±4[b]	163±3[ab]	166±4[a]
2	335±7[a]	309±6[b]	341±6[a]	342±7[a]
3	538±24	495±9	558±13	532±11
4	828±21[a]	716±33[b]	826±18[a]	836±18[a]
5	990±24[ab]	938±17[b]	1030±26[a]	1034±20[a]
6	1116±26[ab]	1067±21[b]	1178±30[a]	1153±30[ab]

a, b, means within rows having different superscripts are significantly different (p<0.05)

energyand protein was found. The higher relative growth rate observed in the first week of the low energy diet could be related to a higher feed intake of those birds. The different effects of energy and protein levels on relative growth rate during the experimental period explain the existence of energy×age and protein×age interactions.

Feed conversion ratio: Breed and diet significantly affected feed conversion ratio (Table 1). Ross birds tended to show a lower FCR than Hybro birds, but this was only significant (p<0.05) at week 6 (Table 6). Male birds showed a lower FCR compared to females at week 6 of age (p<0.05). From day 1 onwards, broiler chickens, which were fed by D2 diet had lowest FCR compared to other groups (Table 7).

Thyroid hormones: Mean plasma T_3 levels are shown in Table 5. Breed and sex had no significant effect on the plasma T_3 concentration but age and diet significantly affected it (Table 1). Plasma concentrations of T_3 decreased and T_4 increased with age in all groups (Table 8 and 9). The effect of energy levels as well as protein levels on plasma T_3 was not significant, but a significant interaction (p<0.001) between energy×protein was observed. Within the high energy diet, birds who received the low protein level showed a tendency for higher T_3 levels in weeks 2, 3 and 4 of age, while on the

Table 6: Weekly feed conversion ratio (g/g) in commercial broilers of two different strains (H: Hybro and R: Ross) and two sexes (F: female and M: male). Values are mean±SEM

	Strain		Sex	
Week	H	R	F	M
1	1.63±0.03[c]	1.62±0.04[cd]	1.61±0.04[c]	1.64±0.03[c]
2	1.58±0.03[c]	1.59±0.04[d]	1.56±0.04[c]	1.61±0.03[c]
3	1.90±0.04[b]	1.83±0.05[bcd]	1.89±0.04[b]	1.83±0.05[bc]
4	1.89±0.08[b]	1.86±0.05[abc]	1.89±0.05[b]	1.87±0.04[b]
5	2.06±0.05[ab]	2.07±0.06[a]	2.04±0.04[b]	2.10±0.07[a]
6	2.15±0.07[a]	1.88±0.06[ab]*	2.29±0.06[a]	1.70±0.05[bc]*

a, b, c, d, means within columns having different superscripts are significantly different (p<0.05). Values followed by asterisk indicate significant differences between the two types of strain and sex at each sampling time (p<0.01)

Table 7: Weekly feed conversion ratio (g/g) in commercial broilers fed four different diets. Values are mean±SEM

	Diets			
Week	D1	D2	D3	D4
1	1.6±0.06[a]	1.4±0.03[b]	1.5±0.03[ab]	1.5±0.05[a]
2	1.5±0.04[a]	1.3±0.03[b]	1.6±0.03[ab]	1.6±0.04[b]
3	1.7±0.08[a]	1.5±0.02[b]	1.8±0.02[ab]	1.8±0.05[a]
4	1.8±0.08[a]	1.6±0.02[b]	1.9±0.03[a]	1.8±0.03[a]
5	2.0±0.03[ab]	1.8±0.02[b]	2.1±0.03[a]	2.2±0.02[a]
6	2.03±0.1[ab]	1.8±0.07[b]	2.3±0.09[a]	2.1±0.07[ab]

a, b, means within rows having different superscripts are significantly different (p<0.05)

Table 8: Plasma T_3 concentration (ng/ml) in commercial broilers fed four different diets. Values are mean±SEM

	Diets			
Week	D1	D2	D3	D4
2	2.6±0.09[ab]	2.9±0.10[a]	2.4±0.10[b]	2.4±0.11[b]
3	2.0±0.10[b]	2.6±0.10[a]	2.5±0.13[a]	2.3±0.12[ab]
4	2.5±0.14	2.6±0.29	2.3±0.14	2.2±0.10
5	1.8±0.1	2.2±0.28	1.7±0.09	1.6±0.11
6	1.6±0.39	1.8±0.09	1.6±0.16	1.5±0.12

a, b, means within rows having different superscripts are significantly different (p<0.05)

low energy diet broiler chickens fed the high protein level showed a tendency for higher plasma T_3 concentrations, probably explaining that presence of a significant energy×protein interaction. Broiler chickens which were fed either D2 or D4 had a significantly (p<0.05) lower plasma T_4 concentration than the other groups at 2, 3 and 4 weeks of age (Table 6). Protein content significantly affected plasma T_4 levels (p<0.05) while energy had no effect (Table 6). No effect of breed on plasma T_3 and T_4 levels were observed.

Growth hormone: The data of mean plasma GH concentrations are presented in Table 10. The overall

Table 9: Plasma T_4 concentration (ng mL^{-1}) in commercial broilers fed four different diets. Values are mean±SEM

	Diets			
Week	D1	D2	D3	D4
2	11±1.10[ab]	7±1.21[c]	13±0.87[a]	9±0.65[bc]
3	14±0.10[a]	10±0.54[b]	14±0.60[a]	11±0.98[ab]
4	15±0.87a	12±0.72[b]	15±0.79[a]	13±0.78[b]
5	17±1.32	14±0.78	15±1.38	15±0.72
6	19±2.44[a]	14±0.81[ab]	16±0.86[ab]	12±0.66[b]

a, b, c, means within rows having different superscripts are significantly different (p<0.05)

Table 10: Plasma GH concentration (ng mL^{-1}) in commercial broilers fed four different diets. Values are mean±SEM

	Diets			
Week	D1	D2	D3	D4
2	41±7.45	29±2.64	39±4.49	32±6.99
3	38±9.16a	27±4.67[ab]	30±3.41[ab]	22±3.08[b]
4	18±1.75[b]	15±3.45[b]	28±5.06[a]	20±3.98[ab]
5	16±2.13[ab]	12±1.32[b]	17±1.91[a]	13±1.60[ab]
6	11±1.97	11±2.18	13±2.13	07±1.01

a, b, means within rows having different superscripts are significantly different (p<0.05)

mean plasma GH levels decreased by age. Sex had a significant effect on plasma GH concentrations (p<0.01), with higher plasma GH levels in male compared to female birds. Broilers were fed the higher protein diet, generally had higher plasma GH levels, which are indicating a central regulation of pituitary function by protein level. No effect of breed on plasma GH levels was observed.

Discussion

Body weight gain, feed efficiency and body fat content of meat-type chickens can be manipulated to a great extent by alterations in dietary E: P ratio (Buyse *et al.*, 1992). In the present study the relative growth was highly dependent on the age of the birds and evidently, male were heavier than female birds. Generally, broilers fed D2 diet (high energy-low protein) tended to show a higher body weight compared with other dietary groups and this was only significant at week 3, 4 and 5. At 42 days of age, there were no significant differences in body weight among broilers fed the different diets. It has been shown that increasing the E:P ratio by means of changing the energy content, protein content or both results in impaired growth rate, poorer efficiency of feed utilization and increased fat accretion, while decreasing the E:P ratio has the opposite effect (Lin *et al.*, 1980; Jones and smith, 1986). Broiler chickens fed with D2 diets in the present study tended to show a better FCR during rearing periods (Table 4). From the study of Buyse *et al.* (1992) it was found that decreasing the crude protein content of isoenergetic diets resulted in depressed body weight gain, impaired feed efficiency and increased abdominal fat deposition. Malheiros *et al.* (2003) observed that chickens fed low crude protein diet showed a reduced body weight, feed intake and FCR compared to those fed low lipid and low carbohydrate diets.

Plasma concentrations of GH decreased with age in all experimental groups and males had higher plasma GH levels than females, regardless of the dietary program used. This age dependent decline and sex dependent plasma GH concentrations were noticed previously (Buyse *et al.*, 1991; Scanes *et al.*, 1992; Decuypere *et al.*, 1993). In the present study broiler chickens, which were fed the higher protein diets, had a tendency for higher plasma GH levels, which is indicating a central regulation of pituitary function by protein level. In a recent study it was documented that genes of the somatotropic axis respond to nutrition differently at the level of transcription (Zaho *et al.*, 2004). To evaluate genotype-diet interaction on growth and the patterns of gene expression of both layer and broiler chickens, layers were fed broiler diet and vice versa. The diet exchange completely reversed the patterns of hypothalamic somatostatin and pituitary GH mRNA expression and the strain differences vanished when the comparison was made on the same diet basis. Furthermore, in this experiment the hepatic GH receptor mRNA decreased in broilers fed with layer diet, but increased in the layer fed with broiler diet (Zaho *et al.*, 2004).

Plasma concentrations of T_3 decreased and of T_4 increased with age in all groups. These observations confirm previous results (Buyse *et al.*, 1991; Kühn *et al.*, 1996). The age related decrease in circulating T_3 levels may result at least partly from the age-related increase in hepatic type III deiodinase activity (increased T_3 degradation) (Kühn *et al.*, 1996). In our study, within the high energy diets, birds fed the low protein diets showed a tendency for higher T_3 levels in weeks 2, 3 of age, while on the low energy diets broiler chickens fed the high protein level showed a tendency for higher plasma T_3 concentrations, probably explaining that presence of a significant energy×protein interaction. Dewil *et al.* (1995) found that birds which were fed the higher energy diet had higher average plasma T_3 levels. In the earlier study it has been found that plasma concentration of T_3 and T_4 were reduced in protein deprived birds compared with those fed adequate protein (Lauterio and Scanes, 1988; Vsilatos Younken *et al.*, 1999; Malheiros *et al.*, 2003). It has been found that chickens fed low protein diet were characterized by the lowest plasma T_4 and uric acid levels compared with chickens fed low lipid diet which had on average higher plasma T_3 and free fatty acid levels (Malheiros *et al.*, 2003). In the present study broiler chickens that were fed either D2 or D4 had a tendency for lower plasma T_4 concentration than the other groups. The lower T_4 values of low protein fed chickens may be indicative for an increased type-I-5´-deiodination. The higher GH as well as T_3 levels at a younger age may suggest the requirement of these hormones for protein synthesis during fast growth phase. It can be concluded that a tendency for lower T_3, higher T_4 and higher plasma GH levels at higher protein diets may support the idea that the high circulating concentration of endogenous GH at younger age may exert a maximal inhibitory effect on T_3 degradations by a dual positive and negative effect on deiodinase type I and type III activity, respectively.

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