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Early lysine deficiency in young broiler chicks

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The carry-over effect of a pre-starter diet (0 to 3 days of age) deficient in lysine on subsequent growth and body composition (3 to 10 days) was examined in two experiments on male broiler chicks raised in cages. In experiment 1, lysine deficiency was applied from 3 to 10 days after providing a balanced pre-starter control feed (D+, 1.40% lysine) or a lysine deficient feed (D−) during the first 3 days. Three levels of deficiency (A = 0.63%, B = 0.72%, C = 0.82%) were tested. Growth and feed intake were higher in D+ than in D− chicks ($P < 0.001$*). However, the feed conversion ratio from 3 to 10 days of age was higher in D+ chicks (*$P < 0.001$*); pre-starter and starter feeds interacted (*$P < 0.04$*) with the feed conversion of treatment D−/A = 2.07 being better than treatment D+/A = 2.61 (*$P < 0.05$*). This suggests that chicks deficient from hatching exhibit a relatively lower sensitivity to lysine deficiency than chicks started on a control diet. In experiment 2, performance, slaughter parameters and body composition were analysed at 3 and 10 days of age, in chicks having received a lysine deficient feed (D0, 0.72% lysine), a control feed (D+, 1.40% lysine) or having been pair fed with control feed adjusted to D0 intake (PF) from 0 to 3 days of age, and then fed D0 ad libitum from 3 to 10 days of age. At 3 days, PF chicks had a higher body weight (*$P < 0.05$*) than D0, and thus a better feed conversion. Body composition in relative values was little or not affected by dietary treatments, but the breast muscle weight at 3 days was higher in D+ and PF chicks compared with D0 (*$P < 0.05$*) and this effect was even accentuated at 10 days of age. The present work confirms that early nutrition can have subsequent consequences on the adjustment of fast growing broiler chicks to their nutritional conditions. It also suggests that breast muscle development is a more reactive parameter than whole body composition in this kind of experiments.*

Keywords: broilers, deficiency, growth, lysine, starter diet

Introduction

Amino acids requirements of broilers have been extensively studied, as well as related factors of influence, such as sex, age, heat stress, energy concentration, and interactions with crude protein level (e.g. Baker and Han, 1994; Vazquez and Pesti, 1997; Garcia *et al*., 2006). It has been identified that the optimal levels of amino acids were not the same for the maximisation of weight gain, feed conversion or breast muscle development (Pesti and Miller, 1997). Moreover, as early as 1965, Dean and Scott underlined the importance of studying the specific case of young animals.

Early nutrition of fast growing broiler chicks immediately after hatching has been showed to affect their development over several days to a few weeks (Bigot *et al*., 2003; Noy and Sklan, 1997; Quentin *et al*., 2005). Compensatory growth after early feed restriction has been showed to modify the amino acid requirement (Plavnik and Hurwitz, 1989). However, the majority of experiments studying the amino acid and protein nutritional requirements start after 1 week of age. This eliminates a very critical period of the life of the chicks during which the residual vitellus (approx. 15% of body weight at hatch) is resorbed, the digestive tract starts a tremendous and essential development (Noy and Sklan, 1997) and most regulatory metabolic loops, including those involved in muscle satellite cells proliferation, are completed (Halevy *et al*., 2000; Bigot *et al*., 2001).

In a recent series of work, Noy and Sklan (2002) and Sklan and Noy (2003) reconsidered the protein nutrition during the 1st week post hatching and observed for

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instance that the effect of some essential amino acid levels on body weight was detected at 7 days of age but not at 4 days. Evidence for a specific sensitivity of the protein turn-over in breast muscles to the nutritional status has been demonstrated in broiler chickens selected on breast meat yield (Tesseraud *et al.*, 1999 and 2001). A lysine deficiency greatly increases the fractional rate of proteolysis in this muscle, more than in the liver at 2, 3 and 4 weeks of age (Tesseraud *et al.*, 1996). From 0 to 4 days of age the weight of the *pectoralis major* muscle increases from 0.5 to almost 3 g (Bigot *et al.*, 2003). Given the major economic value of breast muscle development in broiler chickens and the relatively low contribution of the starter diet in the feeding cost, the effects of the post-hatching nutritional status deserve further consideration.

The present research evaluated the effect of the first feed eaten post hatching (from 0 to 3 days of age) on subsequent (3 to 10 days of age) growth and body composition variation when lysine deficient diets are fed. Lysine deficiency is taken as a model to answer the question: is the pre-starter (0 to 3 days) feed able to change subsequent nutritional responses of chicks to amino acid imbalance?

Material and methods

Two experiments were conducted at the UR83 Recherches Avicoles, Nouzilly, France, using the same facilities, diets and genotype (Ross PM3). Both experiments studied the carry-over effects of an early (0 to 3 days of age = 'pre-starter') lysine deficiency on subsequent responses to lysine deficient diets from 3 to 10 days of age (= 'starter'). Experiment 1 compared the effects on feed intake and growth of three dietary levels of lysine (A = 0.63, B = 0.72 and C = 0.82%) from hatching (D−) or after receiving a balanced control feed for 3 days (D+). Experiment 2 analysed the effects on early body composition at 3 and 10 days of age, of a pre-starter deficient feed (D0) compared with a balanced control (D+) and to a group of chicks pair fed with the control feed at the feed intake levels of D0 counterparts during the pre-starter period of 3 days (PF).

Experimental feeds

The same basal feed (maize/wheat/soya-bean meal/maize gluten meal) was supplemented with synthetic amino acids (DL-methionine, L-valine, L-isoleucine, L-arginine, L-tryptophan and L-threonine) in order to obtain a monodeficiency in lysine (0.63%) in a 13 MJ apparent metabolisable energy (AME) per kg – 240 g/kg of crude protein (CP) starter diet (Table 1). Arginine level was adjusted to obtain a constant ratio to lysine in order to avoid interactions between these amino acids.

Basal feed A was supplemented with 0.13% (diet B), 0.27% (diet C), or 1.08% (control) of L-lysine HCl. At increasing levels of additional L-lysine HCl, nitrogen and ionic balances were maintained by varying glutamic acid, starch, sodium bicarbonate and salt levels. All feeds were mashed.

Experimental procedures

The same battery of 96 cages located in an environmentally controlled room was used in both experiments. Feeders were designed to offer an easy access to the feed while avoiding feed spoilage by the chicks and contamination of feeds by excreta. Lightning was continuous and the environmental temperature was maintained at 32°C during the 1st week and decreased to 30°C until the end of the experiment. The unused cages of the battery were filled with non-experimental birds to homogenise the environment.

Table 1 *Composition and nutrient content of the experimental feeds*

	Feed			
	A	B	C	Control
Composition (%)				
Maize	40.00			
Wheat	20.00			
Soya-bean meal, 48%	9.05			
Maize gluten meal	18.00			
Wheat starch	2.51	2.38	2.24	1.44
Colza oil	1.68	1.75	1.81	2.20
Calcium carbonate	1.20			
Dicalcium phosphate	2.10			
$NaHCO_3$	1.00	1.05	1.11	1.43
Salt	0.30	0.28	0.26	0.14
L-glutamate	2.40	2.24	2.08	1.12
L-lysine HCl	0.00	0.13	0.27	1.08
L-arginine	0.30	0.36	0.42	0.78
DL-methionine	0.10			
L-valine	0.30			
L-isoleucine	0.30			
L-tryptophan	0.06			
L-threonine	0.10			
Vitamins[†]	0.50			
Trace minerals[‡]	0.15			
Total (%)	100	100	100	100
Apparent metabolisable energy (MJ/kg)	13.17	13.17	13.17	13.17
Crude protein[§] (%)	23.7	23.9	24.0	23.2
Lysine[§] (%)	0.63	0.72	0.82	1.40
Methionine + cystine[§] (%)	0.85	0.85	0.85	0.84
Valine[§] (%)	1.25	1.24	1.27	1.26
Isoleucine[§] (%)	1.10	1.09	1.12	1.11
Arginine[§]	1.18	1.20	1.28	1.56
Tryptophan[§]	0.22	0.23	0.23	0.23
Threonine[§]	0.80	0.80	0.80	0.79
Ca (%)	1.03	1.03	1.03	1.03
Available P (%)	0.45	0.45	0.45	0.45

[†] Provides mg/kg of diet: retinol, 3; cholecalciferol, 0.069; alpha-tocopherol, 6.7; menadione bisulphate, 2; riboflavin, 6; Ca pentothenate, 15; niacin, 30; folic acid, 1; cyanocobalamin, 0.01; thiamine mononitrate, 2; choline chloride, 200.
[‡] Provides in mg/kg of diets: Mn, 75; Zn, 60; Fe, 25; Cu, 3; I, 1; Se, 0.02.
[§] Measured value.

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In experiment 1, 168 Ross PM3 day-old male chicks were identified and randomly assigned to 84 cages (two chicks per cage) in 12 blocks of seven neighbouring cages. Within one block, each cage was allocated to one of the seven experimental treatments. This resulted in 12 replicates per treatment. The order of treatments was randomised from one block to another. All feed and water were provided *ad libitum* throughout the experiment. One treatment consisted of the control feed given from 0 to 10 days of age. The six other treatments were distributed as a 2×3 factorial design: two pre-starter (0 to 3 days) feeding conditions: D+ = control or D− deficient feeds (A, B or C) × three starter (3 to 10 days) deficient feeds: A, B and C.

In experiment 2, 192 ROSS PM3 day-old male chicks were identified and randomly assigned to 64 cages (three chicks per cage) in 16 blocks of four neighbouring cages. Within one block, each cage was allocated to one of the four experimental treatments. This resulted in 16 replicates per treatment. The order of treatments was randomised from one block to another. One treatment consisted of the control feed given from 0 to 10 days of age. In the D0 treatment, chicks received feed B from 0 to 10 days of age. In the D+ treatment, chicks received the control feed from 0 to 3 days of age and then feed B from 3 to 10 days of age. Control, D0 and D+ chicks were fed *ad libitum* throughout all the experiment. In a fourth treatment (pair fed, PF), the chicks received the control feed and were pair-fed (from 0 to 3 days of age) based on the feed intake of the D0 counterparts. PF chicks received once daily the amount of feed consumed the previous day by the D0 group +10 to 20% to take into account the evolution of feed consumption at that age. From 3 to 10 days of age, PF chicks received feed B *ad libitum* like the D0 and D+ chicks.

Measurements

The feed intake was measured daily and the chicks were weighed at 0, 3 and 10 days of age at 0900 h. In experiment 2, half of the blocks ($n = 8$) were used for breast muscle dissection and the other half ($n = 8$) for carcass analyses. At 3 days of age, one chick per cage (average body weight of the cage) was sacrificed. The breast muscle (*pectoralis major* muscles left and right) was carefully dissected (Bigot *et al.*, 2003) and immediately weighed (0.01 g). Prior to carcass analyses, the head, legs, digestive tract including the liver and the pancreas (from the proventriculus to cloaca), were removed, weighed, ground and freeze dried. These samples were then analysed in CIRAD (Montpellier, France) for water (103°C until constant weight), total minerals (4 h at 550°C), nitrogen by the Kjeldahl method, and total lipid content by extraction with petroleum ether after acid hydrolysis. At 10 days of age, the same procedures were repeated with the two remaining chicks in each cage.

Nitrogen and amino acid contents of the experimental feeds were analysed by AJINOMOTO EUROLYSINE laboratory (Amiens, France) using Dumas method (AFNOR NFV 18-120) for nitrogen, AFNOR XPV 18-114 for tryptophan and directive 98/64/CE for all the other amino acids.

Statistical analyses

All measurements were averaged per cage (cage = experimental unit). Body weight, weight gain, feed intake and feed conversion (feed intake/weight gain) were analysed by ANOVA. In both experiments, the control treatment, exhibiting much higher values, was systematically excluded from the ANOVAs in order to maintain variance homogeneity among treatments. In experiment 1, the six remaining treatments were analysed by one-way ANOVA for parameters measured at 3 days (pre-starter: four treatments (A, B, C, D+)) and by two-way ANOVA for parameters measured at 10 days (pre-starter: two treatments (D−, D+) × starter: three treatments (A, B, C)). Linear regressions between weight gain and feed intake of the chicks from 3 to 10 days of age were compared for D− and D+ treatment groups and the slopes of the regression lines were compared by *t* test ($P < 0.05$).

In experiment 2, growth, feed intake, feed conversion, and carcass composition of the same eight blocks were analysed by one-way ANOVA (no. of repetitions = 8). Breast muscle weight, expressed as % of body weight, was analysed by one-way ANOVA for the other eight blocks. Treatment means were compared two by two by Newman and Keuls multiple range test ($P < 0.05$).

Results

No mortality occurred during the experiments. Animal fed the control feed exhibited adequate growth performance, although average daily gain (ADG) was lower in experiment 2 – possibly due to lighter chicks at hatching.

Experiment 1

Lysine deficiency reduced ($P < 0.001$) body weight and feed intake at 3 days of age (Table 2a). The control (D+) resulted in higher values than any of the levels of lysine deficiency (A, B, C). Within lysine deficient treatments, differences were significant ($P < 0.05$) between feed A (0.63% lysine) and feed C (0.82% lysine). The feed conversion improved with each increment of lysine. From 3 to 10 days of age (Table 2b), the control group was much heavier than any of the deficient groups, with 244.7 g *v.* 145.2 g for D+/C, the highest of the lysine deficient groups. The feed intake and the weight gain were increased at higher lysine levels ($P < 0.001$). However, there was an interaction ($P < 0.04$) between the pre-starter and starter feeding conditions indicating a lower feed efficiency in D+/A chicks compared with D−/A chicks. This interaction is further illustrated by the regression lines between feed intake and weight gain for that period (Figure 1). At similar feed intake levels, D− chicks gained more weight than their D+ counterparts, as evidenced by the significant difference between the constant terms of the regression (-35.4 ± 7.3 *v.* -12.0 ± 7.3, $P < 0.01$). However the slope of the regressions did not differ significantly (0.83 ± 0.04 *v.* 0.70 ± 0.04, $P = 0.113$).

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Table 2 *Experiment 1: mean body weight, feed intake and feed conversion of broiler chicks at 3 and 10 days of age depending on the pre-starter and starter lysine level in their diet (see Table 1)*

(a) Period 0 to 3 days. Effect of pre-starter lysine level

	Pre-starter feed 0 to 3 days				ANOVA	
	D + (control)	A	B	C	Pre-starter	s.e.
No. of repetitions	48	12	12	12	$P <$	
Body weight at hatch (g per chick)	42.4	41.5	42.1	41.4	NS	0.61
Body weight at 3 days (g per chick)	80.1^{c}	56.2^{a}	59.8^{ab}	62.6^{b}	0.001	0.98
Feed intake 0 to 3 days (g per chick)	31.2^{c}	19.4^{a}	20.8^{ab}	22.7^{b}	0.001	0.48
Feed conversion 0 to 3 days (g/g)†	0.83^{d}	1.33^{a}	1.19^{b}	1.08^{c}	0.001	0.03

$^{a-d}$ Means followed by different superscript are significantly different ($P < 0.05$). NS = not significant at $P > 0.05$.
† Feed conversion from 0 to 3 days is calculated as [(feed intake 0 to 3 days) / (body weight at 3 days − body weight at hatch)].

(b) Period 3 to 10 days. Effect of pre-starter and starter lysine levels

	Pre-starter feed (0 to 3 days)							ANOVA			
	Control	D- (deficient, pooled)‡			D + (control)						
Starter feed (3 to 10 days)	Control	A	B	C	A	B	C	Pre-starter	Starter	Inter-action	s.e.
No. of repetitions	12†	12	12	12	12	12	12	$P <$	$P <$	$P <$	
Body weight at 10 days (g per chick)	244.7	86.6^{a}	97.5^{b}	119.7^{cd}	116.0^{c}	129.1^{d}	145.2 e	0.001	0.001	NS	3.58
Weight gain 3 to 10 days (g per chick)	163.5	30.3^{a}	37.8^{a}	57.0^{bc}	35.1^{a}	50.2^{b}	65.1^{c}	0.001	0.001	NS	2.89
Feed intake 3 to 10 days (g per chick)	211.4	62.0^{a}	72.3^{ab}	96.1^{cd}	89.3^{bc}	101.8^{d}	118.4^{d}	0.001	0.001	NS	3.71
Feed conversion 3 to 10 days (g/g)	1.30	2.07^{b}	1.94^{b}	1.74^{b}	2.61^{a}	2.06^{b}	1.88^{b}	0.001	0.001	0.04	0.09

$^{a-d}$ Means followed by different superscript are significantly different ($P < 0.05$). NS = not significant at $P > 0.05$
† Not used in the ANOVA analysis due to the too high difference of control with other treatments (see text).
‡ Data from the three lysine-deficient diets (A, B, C) were pooled for statistical analysis of period 3 to 10 days.

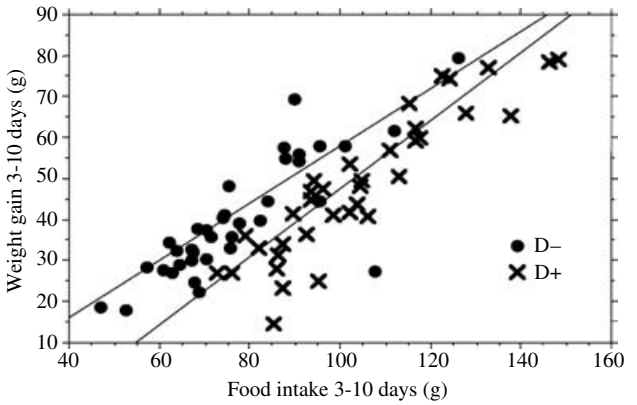

Figure 1 Experiment 1: linear regression of body weight gain (g per chick) to feed intake (g per chick) from 3 to 10 days of age of chicks receiving diets deficient in lysine depending on the lysine level of the pre-starter (0 to 3 days) diet: D− deficient, D+ balanced.

Experiment 2

Lysine deficiency reduced body weight ($P < 0.001$) and feed intake ($P < 0.002$) at 3 days of age (Table 3). For the same feed intake, PF chicks exhibited an intermediate body weight at 3 days, 5.5 g lower than D+ ($P < 0.05$), but 7.0 g higher than D0 ($P < 0.05$). The feed conversion (0 to 3 days) of PF chicks was equal to that of D+ chicks and 30% lower than the feed conversion of D0 chicks ($P < 0.001$). From 3 to 10 days of age, with the same feed (B) deficient in lysine, the difference in feed intake and weight gain of D0, D+ and PF were not significant, but the feed conversion was significantly higher in PF chicks compared with D+ and D0 ($P < 0.005$).

Conversely to growth and feed efficiency, body composition was little affected by the dietary treatments at this early age. Differences in absolute values (Table 3) reflected the body weight variations for each analysed component of the body at 3 and 10 days of age for D+ compared with D0 and PF. In relative values (% body weight or % carcass DM), the dietary effects were not significant (Figure 2a and b) except for the protein content in the carcass at 10 days where PF and

Table 3 *Experiment 2: mean body weight, feed intake, feed conversion and body composition of broiler chicks at 3 and 10 days of age depending on the pre-starter lysine dietary status: D0 = deficient (diet B), D+ = balanced, PF = balanced pair fed to the feed intake of D0 chicks*

	Group				ANOVA	
	Control†	D0	D+	PF‡		
Pre starter: 0 to 3 days		Diet B	Control	Control		
Starter: 3 to 10 days	Control	Diet B	Diet B	Diet B	$P <$	s.e.
Performance (g per chick) $n = 16$						
Body weight at hatch	34.8	35.3	36.1	35.4	NS	0.07
Body weight at 3 days	64.0	53.0[a]	64.5[c]	60.0[b]	0.001	1.15
Body weight at 10 days	184.6	86.1[a]	96.0[b]	85.4[a]	0.05	3.04
Weight gain 3 to 10 days	120.5	33.0	31.6	25.3	NS	2.59
Feed intake 0 to 3 days	26.2	21.2[a]	26.8[b]	21.4[a]	0.002	1.00
Feed intake 3 to 10 days	182.8	75.8	85.3	77.8	NS	3.36
Feed conversion 0 to 3 days	0.99	1.31[b]	1.00[a]	1.00[a]	0.001	0.03
Feed conversion 3 to 10 days	1.53	2.38[a]	2.75[a]	3.18[b]	0.005	0.14
Body composition at 3 days (g per chick) $n = 16$						
Body weight after bleeding	56.2	47.7[a]	58.8[b]	49.5[a]	0.001	1.14
Head + legs	9.9	9.3[a]	10.4[b]	9.6[a]	0.01	0.22
Digestive tract	14.5	10.5[a]	15.0[b]	11.2[a]	0.001	0.30
Water in eviscerated carcass	24.0	20.5[a]	25.0[b]	21.8[a]	0.001	0.76
Ashes in eviscerated carcass	0.60	0.55[ab]	0.66[b]	0.55[a]	0.05	0.03
Protein in eviscerated carcass	4.06	3.63[a]	4.30[b]	3.60[a]	0.02	0.17
Lipids in eviscerated carcass	1.65	1.74[ab]	1.92[b]	1.54[a]	0.05	0.10
Body composition at 10 days (g per chick) $n = 8$						
Body weight after bleeding	179.9	83.0	92.3	82.3	NS	3.67
Head + legs	20.5	13.4	14.2	13.5	NS	0.44
Digestive tract	32.2	17.0[a]	19.4[b]	16.9[a]	(0.06)	0.76
Water in eviscerated carcass	87.1	36.8	41.2	36.1	NS	1.65
Ashes in eviscerated carcass	3.06	1.37[a]	1.70[b]	1.46[ab]	0.04	0.08
Protein in eviscerated carcass	19.1	7.21[a]	8.75[b]	7.62[a]	0.02	0.32
Lipids in eviscerated carcass	16.2	5.98[a]	7.24[b]	5.91[a]	0.04	0.36

[a–c] Means followed by different superscript are significantly different ($P < 0.05$ unless specified) NS = not significant at $P > 0.05$.
† Not used in the ANOVA analysis due to the too high difference of control with other treatments (see text).
‡ Pair fed the same amount of control feed as D0 chicks of the same block from 0 to 3 days.

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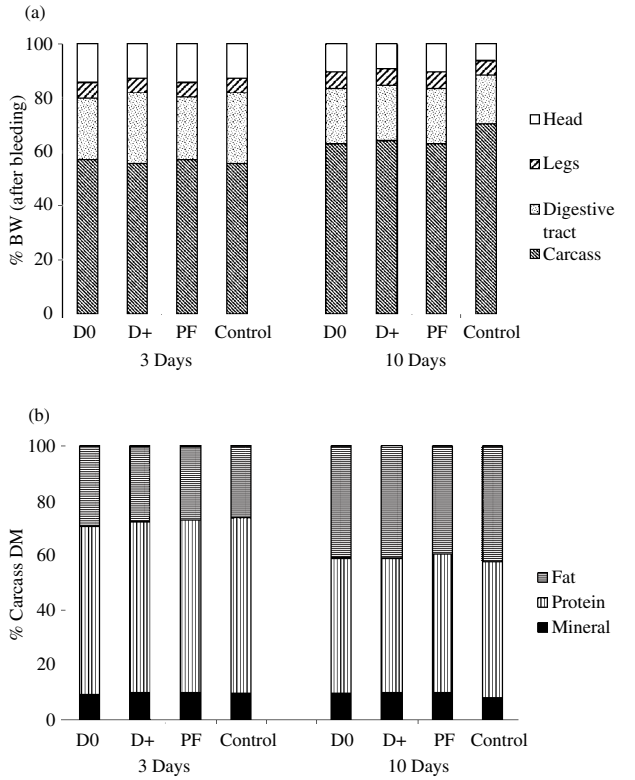

Figure 2 Experiment 2: average slaughter parameters and body composition at 3 and 10 days of age depending on the lysine level in the pre-starter (0 to 3 days) diet: D0 = deficient, D+ = balanced, PF = D+ pair fed to the feed intake of D0 chicks, control. $n = 8$. (a) Slaughter parameters % of body weight (BW) after bleeding. (b) Chemical composition of carcass dry matter (DM).

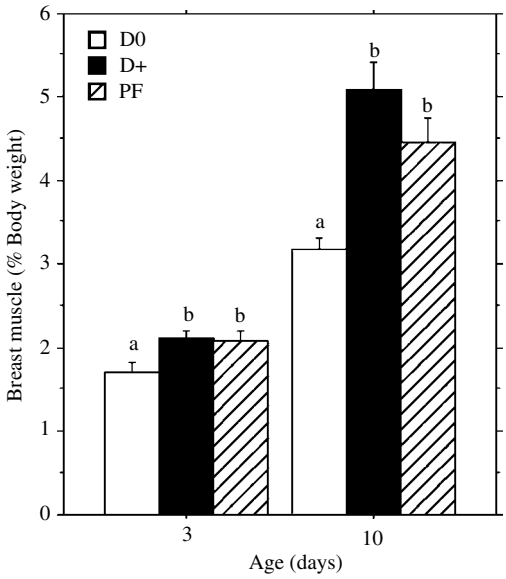

Figure 3 Experiment 2: mean (± s.e.) breast muscle weight (% body weight) at 3 and 10 days of age depending on the lysine level in the pre-starter (0 to 3 days) diet: D0 = deficient, D+ = balanced, PF = D+ pair fed to the feed intake of D0 chicks. [a,b] Values with different letters differ significantly ($P < 0.05$); $n = 8$.

D+ differed significantly ($P = 0.002$). Even the control chicks, which exhibited a growth rate four times higher than the other chicks from 3 to 10 days of age, showed relatively minor variation in the composition of eviscerated carcass at 10 days of age.

Chicks fed the control diet from 0 to 10 days of age had on average 2.19 ± 0.14% breast muscle at 3 days of age and 8.57 ± 0.18% at 10 days of age. The breast muscle growth was significantly decreased by lysine deficiency and the effect of the pre-starter diet was even accentuated from 3 to 10 days of age (Figure 3). Pair fed chicks showed similar breast growth compared with D+ and higher than D0 ($P < 0.05$).

Discussion

During their first 3 days of life broiler chicks receiving a balanced diet doubled their body weight. Both experiments confirmed that the dietary amino acid balance, namely the lysine level in the present research, can strongly modulate this early development (Sklan and Noy, 2003; Quentin *et al.*, 2004 and 2005). Despite the resorption of the vitellus reserves during that period (Bigot *et al.*, 2003) and the development of digestive capabilities (Noy and Sklan, 1997 and 2002), early growth can be decreased to 38% of the control if the feed contains only 0.63% lysine. In contrast, the composition of the tissues deposited seemed to be relatively less affected by the tested levels of lysine at that age.

In experiment 1, we observed a reduced feed intake (62% of controls for 0.63% lysine), which may partly explain the reduction of early growth due to lysine deficiency. Early intake has been demonstrated to be a determining factor for the initiation of growth (Bigot *et al.*, 2001), digestive functions (Smirnov *et al.*, 2004), and further muscular development (Bigot *et al.*, 2003) in young broiler chicks. Lysine level in the present work clearly showed effects on feed intake (experiments 1 and 2) and on body and muscle growth, particularly in the breast muscle (experiment 2). Early response of broiler chickens to amino acid deficiencies have been demonstrated in older chickens. At 8 days of age, broiler chicks offered a 0.63% lysine diet reduced their feed intake to 60% of the controls 6 h after the change of feed (Picard *et al.*, 1993). Such adjustments can hardly be the consequence of major body composition changes even if a change in free amino acids in the muscle (Larbier and Picard, 1977) or in protein synthesis (Kita *et al.*, 1996; Yaman *et al.*, 2000) can be detected over a short period of time. Feed intake adjustment was not the only cause of the reduction of early growth as demonstrated in the second experiment with the pair fed chicks. At 3 days, only approximately 40% of the weight gain reduction could be attributed to feed intake variation, while 60% presumably was due to the lysine deficiency. However, the period of distribution was probably too short (3 days) and the number of repetitions might have been too small (8) to measure precisely the

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effects of pair feeding a balanced diet on the exact tissue deposition of chicks at 3 days of age under our conditions. Further research might consider repeating this approach on larger samples with simultaneous evaluation of protein turn-over.

Lysine deficiency from 0 to 3 days of age had a carry-over effect on subsequent development of chicks in both experiments. Paradoxically in appearance, the deficient pre-starter feeds seemed to have decreased the sensitivity of chicks to a lysine deficiency from 3 to 10 days of age. This was especially measurable on feed conversion in experiment 1. In experiment 2, the difference in feed intake between D0 and D+ was not significant ($P = 0.055$), possibly because the lysine restriction was more moderate than lowest level in experiment 1. However the comparison between PF and D0 showed that for a similar feed intake at 0 to 3 days, animals with a lysine deficiency during this early period had a lower feed conversion from 3 to 10 days ($P = 0.003$). Several reasons might be proposed to explain this surprising observation. Firstly there might be an effect of body weight on the maintenance requirements of chicks at this early age. Little is known on the partition between growth and maintenance in chicks (Sklan and Noy, 2005) and on the cost of the turn-over of muscular protein, i.e. increased protein breakdown, with a lysine deficiency (Tesseraud *et al*., 1996) at this early age. In the present studies, as in other published work (Sklan and Noy, 2004), the body composition was little affected by the diet at this age. As the CP content of the feeds was optimal and constant, only the limiting amino acid level might be responsible for the observed variation and not the interactions between the protein content of the diet and the limiting amino acids (Sklan and Plavnik, 2002; Sklan and Noy, 2003). Since heat production is mainly correlated with the rate of protein accretion (Macleod, 1997), which is strongly associated with the first-limiting factor, a second explanation might be that consuming a low lysine diet, when the chick is not physiologically able to complete thermoregulation, could interfere with set points involved in the control of energy expenditure. A third explanation for the carry-over effect that we recorded might be found on indirect consequences of the low intake of a lysine deficient diet on the development of digestive functions (Noy and Sklan, 2002; Bigot *et al*., 2003; Smirnov *et al*., 2004). It is noteworthy that carry-over effects of amino acid balance have not always been reported in more practical research (Skinner *et al*., 1992). As demonstrated at a later age, factors such as feathering and environmental temperature (Zarate *et al*., 2003a and b) or lysine/arginine ratio in the feed (Labadan *et al*., 2001; Chamruspollert *et al*., 2002) might interfere with the amino acid/energy balance in very young chicks and indirectly act on the variations of feed conversion from 3 to 10 days of age in the present work.

The development of the pectoral muscle seems specifically involved in the immediate and delayed responses to lysine deficiency in young chicks. As demonstrated at later ages early nutrition can durably modulate breast muscle growth (Bigot *et al*., 2003). The effects of genetic selection on differential muscular growth might be a reason for this specific sensitivity of the pectoral muscle to lysine deficiencies (Tesseraud *et al*., 1999; Quentin *et al*., 2005). In our results, chicks with lysine deficiency between 0 and 3 days of age had significantly lower breast muscle (% body weight) than chicks pair fed to the same feed intake, which suggests that the lysine balance has a more important effect than the feed intake. Better understanding of the long-lasting effects of amino acid imbalances on pectoral muscle growth might arise from a more careful determination of the early dynamics of satellite cells and of the changes in expression (RNA and protein) or activation of several metabolic markers involved in protein metabolism such as ribosomal capacities in the pectoral muscle of fast growing broiler chicks (Quentin *et al*., 2003). In avian species, as in mammals, amino acid availability can regulate protein synthesis and gene expression (Kimball and Jefferson, 2002; Tesseraud *et al*., 2003). Short- and longer-term adaptation of protein deposition in the breast muscle to the diet is a major topic of research for broiler chickens. The best applied models to approach this complex but essential problem are early nutrition and sequential feeding (Bouvarel *et al*., 2004). However, the effects observed with the very low levels of lysine studied in the present work might not be true for less deficient diets closer to practical feed formulation and this definitely requires confirmation.

The present research confirmed that early nutrition can have subsequent consequences on the adjustment of fast growing broiler chicks to their nutritional conditions. Specific studies focus on the breast muscle development of broiler chicks might be fruitful in better understanding short- and long-term effects of amino acid supplies.

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