The rate of degradation of myofibrillar proteins of skeletal muscle in broiler and layer chickens estimated by N^{τ}-methylhistidine in excreta

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1. After N^{τ}-methylhistidine (N^{τ}-MH) distribution among the various organs or the tissues was determined in male broiler chickens of 15 d of age, the rates of degradation of myofibrillar proteins in male layer and broiler chickens at different stages of growth were determined by means of N^{τ}-MH.

2. About 75 and 8% of the total N^{τ}-MH in the tissues occurred respectively in skeletal muscle and stomach, and most of the remainder in the intestine and the skin.

3. The rates of degradation of myofibrillar proteins in the male layer and broiler chickens of 21, 42 and 63 d of age were calculated to be 6·1, 4·5 and 2·4%/d (layer) and 5·0, 2·8 and 0·9%/d (broiler) respectively. These calculations involve the assumption that 80% of the total excreted N^{τ}-MH was derived from skeletal muscle.

4. The results strongly indicate that the rapid growth of the broiler chicken is facilitated by the reduced rate of protein degradation.

Growth in animals takes place either by an increase in the rate of tissue protein synthesis or by a reduction in the rate of protein degradation. Therefore, the processes of protein synthesis and protein degradation play equally important roles in the control of protein deposition in animals. The rate of degradation of myofibrillar proteins has been calculated from urinary excretion of N^{τ}-methylhistidine (N^{τ}-MH) in small animals (Young *et al.* 1972; Nishizawa *et al.* 1977), farm animals (Nishizawa *et al.* 1979; Harris & Milne, 1981*a*) and man (Young *et al.* 1973; Long *et al.* 1975).

In the present investigation, N^{τ}-MH distribution among various organs and tissues was determined in broiler chickens, and the rates of degradation of myofibrillar proteins of skeletal muscle in broiler and layer chickens at different stages of growth were estimated.

The present results strongly suggest that rapid growth of broiler chickens originates in the slower rate of body protein degradation.

MATERIALS AND METHODS

Expt. 1. Distribution of N^{τ}*-MH among organs and tissues.* Five commercial male broiler chickens of 15 d of age and weighing 173–205 g were used. The animals were killed by decapitation and the viscera removed quickly and weighed. After removal of the viscera, the skin was removed and weighed. The carcass was weighed and the skeletal muscle excised with a knife and weighed; however, as the skeletal muscle could not be excised completely the bones were boiled for 3 h to remove muscle. The bones were then crushed and a part used for N^{τ}-MH determination. Samples of the organs and tissues were retained for analysis of N^{τ}-MH and stored at −20°.

The N^{τ}-MH content in the samples was analysed by a modification of the method of Nishizawa *et al.* (1978). The samples were weighed into Ehrlenmyer flasks and hydrolysed with 6 M-hydrochloric acid in an autoclave (115°) for 20 h. The samples were completely hydrolysed by autoclaving for 20 h. Almost 100% recovery was demonstrated by treating

Table 1. *Composition of experimental diets (g/kg)*

Diet...	1	2
Ingredients		
Maize meal	870	930
Soya-bean protein	130	70
Amino acids mixture*	10	8
Vitamin mixture † (ml/kg)	1·1	1·1
Mineral mixture‡	3·3	3·3
Analytical composition		
Crude protein (nitrogen × 6·25)	186	148
Crude fat	35	36
Crude fibre	10	13
Metabolizable energy (kJ/g)	13·1	—
N^{τ}-Methylhistidine (mmol/kg)	0·04	0·04

* Contained (g/kg): glycine 380, lysine 330, methionine 150, threonine 70, tryptophan 70.

† Contained (mg/l): retinol 1488, cholecalciferol 24·5, 14·4 α-tocopheryl acetate 7450, 2-methyl-1,4-naphtohydroquinone diacetate 990, riboflavin 1990, pyridoxine 250, cyanocobalamin 9·8, pantothenyl alcohol 1190, biotin 30, pteroylmonoglutamic acid 464.

‡ Contained (g/kg): $CaCO_3$ 213, $CaHPO_4$ 664, NaCl 113, $MnSO_4.4$–$5H_2O$ 6·6, $ZnSO_4.7H_2O$ 3·9, $FeSO_4.7H_2O$ 5·9, $CuSO_4.5H_2O$ 0·23, $NaIO_2$ 0·016.

authentic N^{τ}-MH with the previously-described procedure. The hydrolysates were cooled and passed through filter paper; after hydrochloric acid was removed by evaporation under reduced pressure, the hydrolysates were redissolved in water and evaporated again in the presence of a small amount of sodium hydroxide in order to facilitate removal of ammonia. The residue was solubilized and made up to 25 ml with 0·2 M-pyridine. The resultant solution was passed through filter paper and a portion applied to a resin column (12 × 200 mm, Dowex 50 × 8, pyridine form) to isolate N^{τ}-MH. N^{τ}-MH in the fractions were determined photometrically by a small modification of the method of Ward (1978); in this method most amino acids are not detected. The standard curve was made using N^{τ}-MH solubilized in 1 M-pyridine and the reacted solution of N^{τ}-MH with ninhydrin was allowed to stand for 2 h before measuring optical density.

Expt 2. The rate of excretion of N^{τ}*-MH in chickens.* Commercial male broiler and layer chickens of 21, 42 and 63 d of age were used. The number of birds was ten in each group with the exception of 21-d-old layer chickens (twenty birds). Ingredients, chemical composition and N^{τ}-MH content of the diets are shown in Table 1. The diets were made mainly of maize meal and purified soya-bean protein. Diets 1 and 2 were formulated according to published standards (National Research Council of Agriculture, Forestry and Fishery (Japan), 1974) to contain approximately 200 and 160 g crude protein (nitrogen × 6·25)/kg respectively. Determined crude protein values for both diets were slightly lower than the calculated values. Birds were maintained on the experimental diets for at least 4 d before starting the excreta sample collections. The birds of 21 d of age were fed on diet 1 and the birds of 42 and 63 d of age were fed on diet 2. Excreta samples on the plastic trays were sprayed with boric acid (30 g/l) twice daily at 19.00 and 21.00 hours to minimize microbial destruction of N^{τ}-MH, and collected every day at 14.00 hours using a rubber scraper and a small amount of water. The samples were collected on four consecutive days. The birds were individually housed in wire cages in an air-conditioned room maintained at 23 (SE 1)° under controlled light (14 h light). At the end of the

Table 2. *Expt 1. Distribution of* N^{τ}*-methylhistidine* (N^{τ}*-MH*) *among organs and tissues in 15-d-old chickens*

(Mean values with their standard errors for five birds)

	Fresh wt (g)		N^{τ}-MH (μmol)		
	Mean	SE	Mean	SE	(%)
Skeletal muscle	39·3	2·64	34·1	1·88	57·1
Stomach	7·8	0·31	3·3	0·13	5·7
Heart	1·6	0·04	0·6	0·04	1·1
Liver	6·6	0·09	1·2	0·04	2·2
Skin and feathers*	19·5	1·43	3·1	0·58	5·7
Blood†, bone‡ and head	59·0	2·37	10·9	0·81	19·3
Others	18·0	1·16	5·2	0·22	9·0

* N^{τ}-MH content in feathers, trace.
† N^{τ}-MH content in blood, 0·04 μmol/g.
‡ N^{τ}-MH content in bone, trace.

experimental period, the birds were killed. The carcasses were weighed and skeletal muscles were excised with a knife and weighed. Samples of tissue and excreta were stored at $-20°$ until analysed. N^{τ}-MH contents in the samples and feed were measured as mentioned previously.

Two-way analysis of variance was used to indicate differences between groups and, in the case where the data increased in variability with age or weight of birds, the analysis was performed after log transformation of the data.

RESULTS

Expt 1

The distribution of N^{τ}-MH among the organs and tissues is shown in Table 2. In the case of 15-d-old chickens, skeletal muscle contained 57% of the total body content of N^{τ}-MH. Most of N^{τ}-MH derived from other than skeletal muscle was from stomach, bone and head, skin and feathers and others, and their contributions were 6, 19, 6 and 9% respectively. However, since the N^{τ}-MH contents in the bone and blood were very small, N^{τ}-MH in these tissues seemed to be derived from unremoved skeletal muscle. Therefore, N^{τ}-MH content in the skeletal muscle of 15-d-old chicken appeared to be about 76%.

Expt 2

Body-weight gain and feed conversion ratio of the birds at 21, 42 and 63 d of age are shown in Table 3. Body-weight gain was higher in the broiler than in the layer chickens at all stages of growth. However, although the feed conversion ratio was superior in broiler chickens than in layer chickens at 21 and 63 d of age, the result was reversed at 42 d of age. Weight of muscle and N^{τ}-MH contents in the muscle and excreta are shown in Tables 4 and 5 together with the contribution of feed to the N^{τ}-MH content in excreta. N^{τ}-MH derived from feed was subtracted from the excreted N^{τ}-MH to estimate N^{τ}-MH derived from body tissues, and this was multiplied by 0·8 to give roughly the excreted N^{τ}-MH derived from skeletal muscle. N^{τ}-MH concentration of muscle (μmol/g) was significantly higher in layer chickens

Table 3. *Expt 2. Body-weight gain and feed conversion ratio of layer and broiler chickens at different stages of growth*

(Mean values with their standard errors)

Age (d)	*n*	Final body-wt (g)				Body-wt (g/6 d)				Feed conversion ratio†			
		Layer		Broiler		Layer		Broiler		Layer		Broiler	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
21	20	266	11	445**	13	84	4	156**	10	2·2	0·06	1·9**	0·09
42	10	473	13	1623**	27	120	4	228**	10	2·5	0·06	3·2**	0·13
63	10	798	28	2152**	31	106	15	400**	17	4·7	0·89	2·3*	0·13

Values differ significantly from those for layer chickens: * $P < 0·05$, ** $P < 0·01$.
† Feed intake (g) ÷ weight gain (g).

Table 4. *Expt 2.* N^{τ}*-Methylhistidine* (N^{τ}*-MH*) *content in muscle in layer and broiler chickens at different stages of growth*

(Mean values with their standard errors)

Age (d)	*n*	Skeletal muscle wt (g)				Muscle N^{τ}-MH (μmol/g muscle)				Total muscle N^{τ}-MH (μmol)			
		Layer		Broiler		Layer		Broiler		Layer		Broiler	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
21	20	64·4	2·6	114·7**	3·4	0·745	0·027	0·606**	0·018	48·0	2·0	69·5**	2·7
42	10	124·0	4·9	482·1**	8·1	0·794	0·025	0·662*	0·047	98·5	6·3	319·1**	5·3
63	10	209·2	9·7	706·1**	11·1	0·743	0·019	0·707	0·044	155·4	7·6	499·2**	22·0

Values differ significantly from those for layer chickens: * $P < 0·05$, ** $P < 0·01$.

Table 5. *Expt 2. Origin of* N^{τ}*-Methylhistidine* (N^{τ}*-MH*) *excreted by layer and broiler chickens at different stages of growth*

(Mean values with their standard errors)

Age (d)	*n*	Total excreted N^{τ}-MH (μmol/d)				N^{τ}-MH derived from feed (μmol/d)				N^{τ}-MH derived from skeletal muscle (μmol/d)			
		Layer		Broiler		Layer		Broiler		Layer		Broiler	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
21	20	5·37	0·15	6·89**	0·39	1·72	0·06	2·62**	0·10	2·92	0·15	3·42	0·27
42	10	8·15	0·42	17·88**	0·81	2·72	0·08	6·59**	0·20	4·34	0·32	9·03**	0·61
63	10	8·43	0·60	13·72**	0·90	3·79	0·15	8·40**	0·26	3·71	0·38	4·26	0·64

Values differ significantly from those for layer chickens: * $P < 0·05$, ** $P < 0·01$.

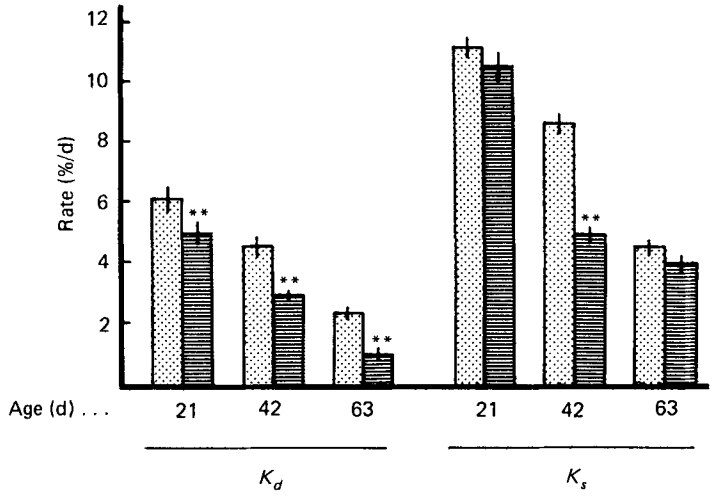

Fig. 1. Expt 2. Rates of muscle protein degradation (K_d) and synthesis (K_s) of layer (▨) and broiler (▤) chickens at different stages of growth. Values are means with their standard errors represented by vertical bars. Values differ from those for layer chickens: ** $P < 0.01$.

than in broiler chickens. The large difference of muscle N^{τ}-MH content between layer and broiler chickens was a result of difference in muscle size.

Rates of skeletal muscle protein degradation and synthesis are shown in Fig. 1; they were calculated by the equations reported by Funabiki *et al.* (1976). The rates of degradation were significantly higher in layer than in broiler chickens. The rates of degradation in the male layer and broiler chickens of 21, 42 and 63 d of age were 6·1, 4·5 and 2·4%/d (layer) and 5·0, 2·8 and 0·9%/d (broiler) respectively. The rates of synthesis were much the same in both breeds of chicken with the exception of 42-d-old chickens in which the rate was significantly higher in layer than in broiler chickens. The rates of synthesis in the male layer and broiler chickens of 21, 42 and 63 d of age were 11·2, 8·7 and 4·5%/d (layer) and 10·5, 4·9 and 3·9%/d (broiler) respectively. These calculations involve the assumption that the rates of protein degradation of all tissues were the same as that of muscle.

DISCUSSION

N^{τ}-Methylhistidine (N^{τ}-MH) is an amino acid produced within tissues by the post-translational methylation of histidine in actin and in myosin of white muscle fibres. When proteins containing N^{τ}-MH are degraded, the amino acid is released but not re-utilized or metabolized, and it is excreted unchanged in the urine. Injected N^{τ}-MH is not altered in its passage through the body, N^{τ}-MH does not charge tRNA for protein synthesis, and the major pool of N^{τ}-MH in the whole body is muscle (Young & Munro, 1978). In chickens, Cowgill & Freeburg (1957) and Saunderson & Leslie (1983) observed that injected N^{τ}-MH, labelled with ^{14}C in the methyl group, was not oxidized nor incorporated into proteins, and most of it was recovered in the excreta. On the basis of these findings it has been suggested that the rate of skeletal muscle protein degradation may be estimated from the excretion of N^{τ}-MH. The potential of the N^{τ}-MH measurement has been quickly realized in clinical studies, but with the obvious importance of lean meat in animal farming practice the technique has a potential role in agricultural research.

However, contractile proteins in non-skeletal muscle tissues may turn over so rapidly that,

although their pools of protein-bound N^{τ}-MH are small, these non-skeletal muscle sources may contribute considerably to the daily N^{τ}-MH excretion in the urine (Millward *et al.* 1980; Wassner & Li, 1982; Millward & Bates, 1983). Nagasawa & Funabiki (1981) have reported that the contributions to urinary excretion of N^{τ}-MH from skeletal muscle, intestine and skin (%) were 75·6, 2·2 and 22·2 respectively in rat, and the fractional degradation rates (K_d) of those tissues (%/d) were 2·87, 5·99 and 8·72 respectively. These results show that the contribution of skin is not negligible. The problem becomes acute if the muscle mass is reduced as a result of muscle wasting. Griggs & Rennie (1983) and Rennie & Millward (1983) have reported that urinary excretion of N^{τ}-MH cannot be used as a measure of skeletal muscle protein degradation.

In most farm animals, muscle comprises a larger proportion of lean body mass than it does in the rat; therefore, even if non-muscle protein turns over at a higher rate than muscle protein, the problem should be less significant than that in the rat. Our results showed that skeletal muscle contained about 76% of the total body N^{τ}-MH in the 15-d-old broiler chicken. Skeletal muscle comprises about 26% of the live weight in the 15-d-old broiler chicken and it comprises about 43% in the 90-d-old broiler chicken (K. Hayashi, Y. Tomita, Y. Maeda, Y. Shinagawa, K. Inoue and T. Hashizume, unpublished results). The blood, bone and head comprise about 28% of the live weight in the 90-d-old broiler chicken, and the N^{τ}-MH content of these tissues is calculated to be about 11%. As mentioned earlier, N^{τ}-MH of these tissues seems to come from unremoved skeletal muscle, thus the N^{τ}-MH content in the skeletal muscle of 90-d-old chickens is expected to be about 84%.

Although there is some evidence that N^{τ}-MH measurement is not always valuable, as mentioned previously, in most normal farm animals it must still be a useful index of the rate of muscle protein degradation, except sheep (Harris & Milne, 1980) and pig (Harris & Milne, 1981*b*).

Macdonald & Swick (1981) reported that the fractional degradation rates in breast-muscle protein of 4-, 6- and 7-week-old-layer chickens were 18, 14 and 13%/d respectively. In their experiment, the rates of protein synthesis were measured by injecting a massive dose of L-[1 $-{}^{14}$C]valine, and rates of protein degradation were estimated as the difference between the synthesis rate and the growth rate of muscle protein. These values are much higher than the present values and the age-difference is much smaller than that in the present study (Fig. 1). However, Macdonald & Swick (1981) stated that there is a wide range of variation in the estimates of the rates of protein synthesis and degradation and, at present, no known radioisotopic technique gives satisfactory rates of degradation in muscle under all conditions.

Studies in young animals have shown that high rates of growth are accompanied by both elevated protein synthesis and degradation (Perry, 1974; Millward *et al.* 1975), a situation which is energy expensive. The present selection of farm animals is based mainly on an increased appetite effect whereas a more efficient deposition of protein, involving both conservation of N and energy, is preferred. However, we are limited in basic information to aid the geneticist, other than to state the ideal of high synthesis and low degradation rates.

Our experiment clearly showed that K_d was smaller in the broiler than in the layer chickens and that fractional synthesis rate (K_s) was much the same in both breeds, with the exception of the 42-d-old chickens where the K_s was significantly lower in the broiler than in the layer chickens. The feed conversion ratio was also superior in the 42-d-old layer chickens than in the 42-d-old broiler chickens, where the value was lower than that reported by Reece & Lott (1983). They reported that the feed conversion ratio of broiler chickens was linear with age over the period 35–55 d of age. We repeated the experiment to check the feed conversion ratio. However, the result was similar to that reported in the present experiment.

The present results strongly indicate that the rapid growth and efficient feed conversion of the broiler chickens is facilitated by not only an increased appetite but also by a lowered rate of protein degradation, and that the rate of excretion of N^{τ}-MH may contribute to the genetic selection of meat animals.

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