

## Efficiency of methionine retention in ducks

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The accretion of methionine and protein as a function of methionine intake was assessed in growing ducks between 22 and 42 d post-hatching. Four graded doses of DL-methionine at 0, 0.5, 1.0 or 1.5 g/kg diet were added to a methionine-limiting basal diet and fed to four replicate groups of four ducks each. The growth and efficiency of food use for growth increased linearly ( $P < 0.05$ ) as a function of methionine intake. The accretion of body protein increased ( $P < 0.001$ ) from 87.5 to 182.2 g, and that of methionine from 1616 to 3125 mg, over the 21 d period as dietary methionine increased. The accretion rate of methionine in the body ( $y$ , mg/d) as a function of methionine intake ( $x$ , mg/d) of ducks fed diets containing supplemental methionine at 0, 0.5, 1.0 or 1.5 g/kg diet from day 22 to day 42 post-hatching gave the regression equation:  $y = -148.86$  (SE 32.558) + 0.312 (SE 0.0384) $X$ ,  $r^2 = 0.8253$ . For protein accretion rate in the body ( $y$ , mg/d) as a function of methionine intake ( $x$ , mg/d), the regression equation was:  $y = -9782$  (SE 2204) + 19.505 (SE 2.5994) $x$ ,  $r^2 = 0.8009$ . There was a linear relationship between methionine ( $y$ , mg/d) and protein ( $x$ , mg/d) accretion in ducks that was described by the equation  $y = 12.757$  (SE 7.4019) + 0.01525 (SE 0.00107) $x$ ,  $r^2 = 0.9355$ . The results of these studies suggest a constant utilisation of methionine over the range 2.4–3.9 g digestible methionine/kg diet, with an efficiency of 31%. Furthermore, the results suggest a quantitative relationship of 15 mg methionine for every gram of protein accretion.

### Amino acids: Ducks: Methionine: Protein: Retention efficiency

The development of accurate models of nutritional requirements for amino acids of necessity calls for a knowledge of maintenance requirements, efficiencies of utilisation of amino acids for protein accretion, and quantitative relationships between the accretion of protein and amino acids. The amount of dietary protein that must be supplied is dependent on the efficiency with which dietary protein is used for body functions. However, the efficiency of dietary protein utilisation is not necessarily equal to that of individual amino acids (Baker, 1991; Chung & Baker, 1992).

Thus, data on dietary protein utilisation provide no knowledge on the efficiency of utilisation of individual amino acids, and there is evidence in pigs, chickens and rats that these efficiencies are not equal between amino acids within species (Gahl *et al.* 1991; Batterham *et al.* 1990; Bikker *et al.* 1994; Adeola, 1995; Baker *et al.* 1996; Sklan & Noy, 2004). Adeola (1995) reported that lysine was retained more efficiently than threonine in pigs. Edwards *et al.* (1997, 1999), and Edwards & Baker (1999), reported that the efficiency of dietary threonine, lysine and methionine retention in young chicks was 82%, 79% and 68%, respectively. Estimates of the efficiency of amino acid retention for ducks are limited. Timmler & Rodehutsord (2003) presented data suggesting that the efficiency of retaining valine was 49% in ducks between 0 and 21 d post-hatching. In an earlier study, Adeola (1998) evaluated tryptophan accretion as a function of tryptophan intake in 42-d-old ducks and observed that the efficiency of tryptophan retention was 21%.

Because of the importance of methionine as a limiting amino acid for non-ruminant animals and the paucity of

estimates of efficiency of methionine utilisation for protein and methionine accretion in ducks, the study reported here was designed to investigate methionine retention in the growing duck.

### Materials and methods

#### General procedures

Purdue University Animal Care and Use Committee approved all housing and handling procedures. White Pekin drakes were obtained from Maple Leaf Farms (Syracuse, IN, USA) 1 d post-hatching and housed in pens (1.52 × 2.44 m) with raised, plastic-coated wire floors (1.52 × 0.81 m) over gutters. The ducklings had free access to a commercial nutrient-adequate duck starter diet and water for 21 d on a regimen of 23 h/d light.

On day 22, the drakes were weighed, wing-banded and divided into experimental groups. An experimental group of twelve drakes served as an initial slaughter group. Furthermore, there were sixteen groups of four drakes each that were assigned to four diets as indicated below. Four graded doses of DL-methionine at 0, 0.5, 1.0 or 1.5 g/kg diet were added to a basal diet. The sixteen groups of ducks were blocked on the basis of weight with four groups per block. Each block of similar weight was then randomly allotted to each of the four diets. Individual body weight and cage food intake were recorded weekly, and access to food and water was provided *ad libitum* for 21 d. The ingredient composition as well as analysed nutrient content of the basal diet are

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presented in Table 1. All amino acids, except methionine, in the basal diet met National Research Council (1994) requirements for 2–7-week-old ducks. As a part of the current study, amino acid digestibility coefficients and N-corrected true metabolisable energy were determined in the basal diet using procedures previously described (Ragland *et al.* 1999). The product of amino acid digestibility coefficient and analysed amino acid concentration was used as the digestible amino acid concentration. Diets containing the graded doses of methionine were formulated by supplementing the basal diet with DL-methionine at the expense of corn starch.

#### Analytical procedures

At the end of the feeding period, feed was withdrawn for 12 h. The ducks were shipped to the Animal Sciences Meat Laboratory where they were electrically stunned and killed. The whole body was subsequently frozen, sliced into strips and homogenized. Samples of homogenates were lyophilized. DM was determined

by drying the samples at 105°C for 24 h. Ether-extractable fat content was assessed by extracting the lyophilized samples in diethyl ether and drying the extract at 105°C for 12 h. The lyophilized samples were burned in a muffle furnace at 550°C for 24 h to determine the ash content. N content was determined by the combustion method using a LECO model FP-2000 Nitrogen Analyzer (LECO, St Joseph, MI, USA).

Amino acid analyses were conducted at the University of Missouri Experiment Station Chemical Laboratory. Samples for amino acid analysis were prepared using 24 h hydrolysis in 6 M-HCl at 110°C under an atmosphere of N. For methionine and cysteine, samples were oxidized in performic acid before acid hydrolysis. Samples for tryptophan analysis were hydrolyzed using Ba(OH)<sub>2</sub>. Amino acids in hydrolysates were determined by HPLC after post-column derivatization (Association of Official Analytical Chemists, 2000).

#### Statistical analysis

Data were subjected to the generalized linear models procedure (SAS Institute, 2003) using the following model:

$$y_{ijk} = \mu + B_i + D_j + E_{ijk}$$

where  $y$  is the response criterion,  $\mu$  is the overall mean,  $B_i$  represents the effect of  $i$ th block ( $i = 1, 2, 3, 4$ ),  $D_j$  represents the effect of  $j$ th diet ( $j = 1, 2, 3, 4$ ) and  $E_{ijk}$  is the error term. Linear and quadratic contrasts were used to examine the relationship between response and dietary methionine. For methionine accretion data, simple linear regression analysis was conducted using the generalized linear models procedure. In the analysis, we regressed the methionine accretion for the mean of four ducks per cage on the average methionine intake to obtain the relationship between accretion and intake of methionine; and the quantitative relationship between methionine and protein accretion was derived from the linear regression of methionine accretion on protein accretion. Mean differences were considered significant at  $P < 0.05$ .

#### Results

The addition of up to 1.5 g methionine to each kilogram of the methionine-deficient basal diet linearly increased ( $P < 0.001$ ) body weight gain and food efficiency of the ducks (Table 2).

**Table 2.** The weight gain, food intake and food efficiency of ducks fed diets containing supplemental methionine at 0, 0.5, 1.0 or 1.5 g/kg diet for 21 d

	Supplemental dietary methionine (g/kg diet)				SEM
	0	0.5	1.0	1.5	
Initial weight (g)	1343	1339	1336	1337	36.76
Final weight (g)***	2171	2565	2705	2859	79.26
Weight gain (g)***††	828	1226	1369	1522	53.23
Food intake (g)	4370	4475	4531	4468	78.84
Gain:food (g/kg)***†	190	275	302	341	11.77

\*\*\* Linear effect at  $P < 0.001$ .

† Quadratic effect at  $P < 0.10$ .

†† Quadratic effect at  $P < 0.05$ .

**Table 1.** Ingredient composition (g/kg) of the basal diet on an as-fed basis†

Ingredient	Composition
Corn	320
Corn gluten meal	160
Sucrose	100
Skimmed milk powder (34% CP)	30
Casein	25
Corn oil	40
Solka floc	30
Crystalline amino acid premix	30
Dicalcium phosphate	16
Limestone	10
Salt	4
Minerals and vitamins‡	3
Corn starch§	232
Total	1000
Analyzed nutrient (g/kg) and energy (MJ/kg)	
Crude protein (N × 6.25)	165.8
N-corrected true metabolisable energy	14.8
Ca	7.5
P	4.8
Ca:P	1.6
Non-phytate P	3.6
Total amino acids	
Arg	10.8
His	4.5
Ile	7.6
Leu	23.4
Lys	12.2
Met	3.7
Cys	7.5
Phe	10.1
Tyr	8.5
Thr	8.4
Trp	1.5
Val	9.2

‡ Composed of the following (per kg diet): retinol 1.65 mg, cholecalciferol 66 mg, DL- $\alpha$  tocopherol 10 mg, menadione sodium bisulfite 438 mg, riboflavin 5.49 mg, D-pantothenic acid 11 mg, niacin 44.1 mg, choline chloride 771 mg, cyanocobalamin, 13.2 mg, biotin 55.2 mg, thiamine mononitrate 2.2 mg, folic acid 990 mg, pyridoxine hydrochloride 3.3 mg, I 1.11 mg, Mn 66 mg, Cu 4.44 mg, Fe 44.1 mg, Zn 44.1 mg, Se 300 mg.

§ DL-methionine replaced corn starch in the test diets.

|| Determined using procedures referenced in Materials and Methods.

Methionine supplementation also resulted in a quadratic effect on body weight ( $P < 0.05$ ) and food efficiency ( $P < 0.10$ ). The increase in body weight gain and food efficiency as a result of adding methionine to the basal diet was not caused by changes in food intake of the ducks as there was no effect on food intake (Table 2). There was a linear increase ( $P < 0.01$ ) in body ash content as supplemental methionine in the basal diet offered to ducks increased from 0 to 1.5 g/kg (Table 3). Whereas ether-extractable fat contents linearly decreased ( $P < 0.01$ ) with added dietary methionine, there was a linear increase ( $P < 0.05$ ) in protein. Most of the amino acids in the body protein except arginine, cysteine, methionine and threonine were unaffected by supplementation with methionine.

The accretion of body DM, ash, ether extract, protein and methionine increased linearly ( $P < 0.001$ ) as supplemental methionine in the basal diet offered to the ducks increased from 0 to 1.5 g/kg (Table 4). The linear regression of body methionine accretion rate ( $y$ , mg/d) against daily digestible methionine intake ( $x$ , mg/d) was described by the line:  $y = -148.86$  (SE 32.558) +  $0.312$  (SE 0.0384) $x$ ,  $r^2 = 0.8253$  (Fig. 1). This indicated that approximately 31 % of digestible methionine intake was deposited in the body of the ducks during the growth period between 22 and 42 d post-hatching.

The daily accretion of protein ( $y$ , mg/d) as a linear function of daily digestible methionine intake ( $x$ , mg/d) was described by the equation:  $y = -9782$  (SE 2204) +  $19.505$  (SE 2.5994) $x$ ,  $r^2 = 0.8009$  (Fig. 2). On the basis of extrapolation of the regression equation to the  $y$  intercept, there was a net daily loss of 9.8 g body protein in ducks at zero methionine intake. Fig. 3 depicts the linear regression of daily body methionine accretion ( $y$ , mg/d) on protein accretion ( $x$ , mg/d). The regression equation,  $y = 12.757$  (SE = 7.4019) +  $0.01525$  (SE = 0.00107) $x$ ,  $r^2 = 0.9355$ , indicated that the accretion of

body methionine increased by 15.3 mg for every 1 g increase in protein accretion.

## Discussion

The responses of ducks to dietary methionine supplementation in terms of body weight gain and the efficiency of conversion of food to body weight in the current study confirms that methionine was the first limiting amino acid in the basal diet. In agreement with a previous study reported by Elkin *et al.* (1986), increasing digestible methionine from 2.4 to 3.9 g/kg feed improved the body weight gain and food efficiency of 22–42-d-old ducks in the current study.

In studies with other amino acids, Oluyemi & Fetuga (1978) observed improvements in weight gain and food efficiency with increasing dietary lysine in ducks, and Chen & Shen (1979) reported improvements in weight gains and food efficiency with increasing dietary arginine in ducks. Wu *et al.* (1984), in a study of the response of 8–20-d-old Mule ducklings to dietary tryptophan, observed a 32 % and 25 % improvement in weight gain and food efficiency, respectively, when dietary tryptophan was increased from 1.3 to 2.8 g/kg feed. In more recent studies with White Pekin ducks, Adeola (1998) and Timmler & Rodehutsord (2003) reported body weight gain and food efficiency responses to dietary tryptophan and valine, respectively. The restriction imposed by the limiting amino acid on the use of other amino acids in the current study was presumably alleviated as dietary methionine increased, hence the observed increase in body weight gain and food efficiency responses to dietary methionine supplementation. The data from the current study demonstrate that dietary methionine did not affect voluntary food intake, and illustrate that the ducks used in the current study grew and gained protein and methionine in response to the quantity of methionine consumed.

**Table 3.** Body DM, ash, ether extract, protein and amino acids of ducks fed diets containing supplemental methionine at 0, 0.5, 1.0 or 1.5 g/kg diet for 21 d

	Supplemental dietary methionine (g/kg diet)				SEM
	0	0.5	1.0	1.5	
DM (g/kg)	396	404	405	402	6.96
Ash (g/kg)†**	58.8	61.2	67.2	70.4	2.72
Ether extract (g/kg)†**	591	565	550	517	12.48
Protein (g/kg)†*	322	327	354	362	12.37
Amino acids (g/kg protein)					
Arg*	70.37	69.89	71.11	71.57	0.416
Cys*	11.24	10.72	11.79	11.77	0.212
His	31.33	31.48	32.01	31.74	0.326
Ile	32.63	32.53	33.08	33.16	0.459
Leu	71.91	71.82	72.70	73.04	0.901
Lys	65.39	64.91	65.93	66.24	0.773
Met*	17.26	16.54	17.00	18.17	0.336
Phe	43.49	43.18	43.91	44.04	0.520
Thr*	38.76	37.50	40.64	40.61	0.614
Trp	7.46	7.46	7.54	7.58	0.089
Val	42.51	42.44	42.97	43.17	0.532

\*Linear effect at  $P < 0.05$ .

\*\*Linear effect at  $P < 0.01$ .

† Ash, ether extract and protein are expressed on a DM basis; protein =  $N \times 6.25$ .

**Table 4.** Accretion of body DM, ash, ether extract, protein and methionine of ducks fed diets containing supplemental methionine at 0, 0.5, 1.0 or 1.5 g/kg diet for 21 d

	Supplemental dietary methionine (g/kg diet)				SEM
	0	0.5	1.0	1.5	
Dry matter (g)***	274.49	403.88	451.59	504.05	25.517
Ash (g)***	16.33	24.67	30.43	35.52	2.185
Ether extract (g)***	162.30	227.40	248.93	261.42	15.803
Protein (g)***‡	87.50	131.61	159.70	182.22	7.573
Methionine (mg)***	1616.3	2263.8	2623.0	3125.0	130.98

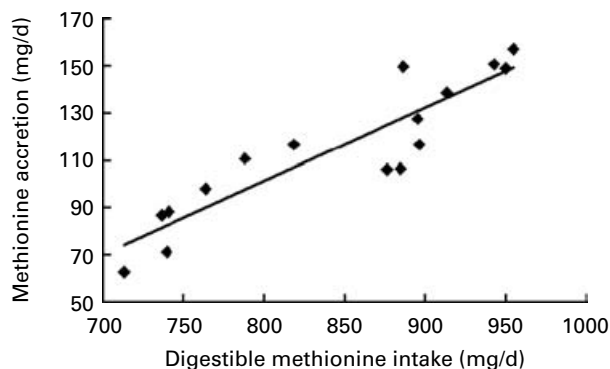
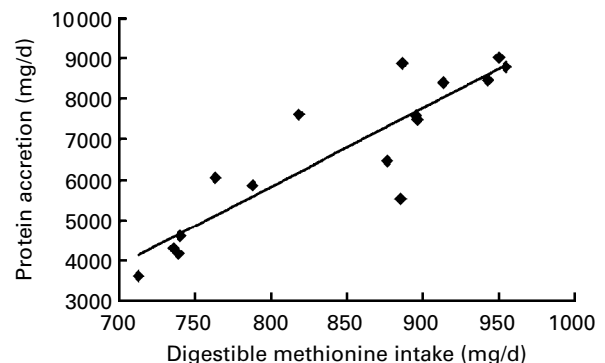
\*\*\* Linear effect at  $P < 0.001$ .‡ Protein =  $N \times 6.25$ .

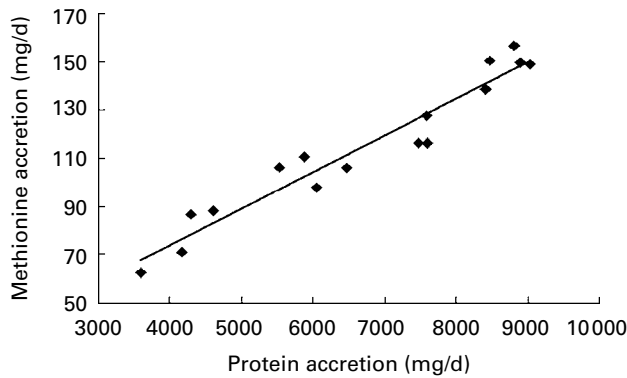
Modelling nutritional needs for amino acids requires accurate information on the efficiency with which amino acids are used for various body processes. The classical protein evaluation methods – biological value and protein efficiency ratio – do not provide the vital information on the efficiency with which individual amino acids are utilized for body processes, and there is hence a need for direct investigation of individual amino acids. There is a considerable difference of opinion regarding whether the above-maintenance efficiency of amino acid utilisation is variable or constant in growing animals fed graded dietary levels (to near-optimal) of a limiting amino acid (Baker, 2004). Data acquired in the current study on methionine and protein retention indicate the following important points: between 2.4 and 3.9 g (near-optimal) digestible methionine/kg diet, the above-maintenance efficiency of methionine retention in White Pekin ducks from 22 to 42 d post-hatching was constant, the above-maintenance efficiency of retaining digestible methionine was 31 %, and at all levels of digestible methionine intake, there was an accretion of 15 mg methionine/g body protein accretion.

Over the years, there has been controversy over the constancy of efficiency of amino acid utilisation for retention and whether diminishing returns occur at amino acid intakes above maintenance level (Heger & Frydrych, 1985; Baker, 1991; Adeola, 1995, 1998; Timmler & Rodehutschord, 2003). To the current author's knowledge, there are no published data on studies considering the efficiency of methionine retention in ducks. The observation

that methionine accretion is a linear function of methionine intake in the current experiment is in agreement with previous work from this laboratory on tryptophan retention in 3–6-week-old ducks (Adeola, 1998). The constancy of efficiency of methionine retention in the present study is of particular interest as it illustrates that the dietary concentration of other amino acids relative to methionine had no pernicious effects on the utilisation of methionine.

Previous studies in 1–3-week-old ducks show a variable above-maintenance efficiency of valine retention (Timmler & Rodehutschord, 2003), suggesting the occurrence of diminishing returns at valine intakes above maintenance level. In earlier studies with rodents, Heger & Frydrych (1985) and Gahl *et al.* (1991) reported a variable efficiency of retention of threonine and lysine. The role of coprophagy in the observations by Heger & Frydrych (1985) and Gahl *et al.* (1991) cannot be quantified and may be responsible for the observations, as Baker *et al.* (1996) suggested. The current data, as well as the lysine retention data of Batterham *et al.* (1990), Adeola (1995) and Heger *et al.* (2002) with pigs, the lysine, isoleucine and valine retention data of Baker (1991) and Baker *et al.* (1996) with chicks, and the data on branched-chain and large-neutral amino acids from Heger *et al.* (2003) with pigs, lend support to the notion that, within a range of limiting amino acid intake that is close to optimal, the above-maintenance efficiency of retention of an individual amino acid is constant.

**Fig. 1.** Methionine accretion rate in the body (mg/d) as a function of daily digestible methionine intake (mg/d) of ducks fed diets containing supplemental methionine at 0, 0.5, 1.0 or 1.5 g/kg diet from day 22 to day 42 post-hatching. The regression equation was  $y = -148.86$  (SE 32.558) +  $0.312$  (SE 0.0384) $x$ ,  $r^2 = 0.8253$ .**Fig. 2.** Protein accretion rate in the body (mg/d) as a function of daily digestible methionine intake (mg/d) of ducks fed diets containing supplemental methionine at 0, 0.5, 1.0 or 1.5 g/kg diet from day 22 to day 42 post-hatching. The regression equation was  $y = -9782$  (SE 2204) +  $19.505$  (SE 2.5994) $x$ ,  $r^2 = 0.8009$ .



**Fig. 3.** Methionine accretion rate in the body (mg/d) as a function of protein accretion rate (mg/d) in ducks fed diets containing supplemental methionine at 0, 0.5, 1.0, or 1.5 g per kg from day 22 to 42 post-hatch. Regression equation was  $y = 12.757$  (SE = 7.4019) + 0.01525 (SE = 0.00107)x,  $r^2 = 0.9355$ .

Linear regression relating methionine accretion to intake indicated that the efficiency of methionine retention in ducks was 31%. The literature is replete with estimates of amino acid retention efficiency in pigs and broiler chickens. In experiments with growing pigs, Batterham (1994) reported efficiency values for lysine, threonine, methionine and tryptophan of 75%, 64%, 45% and 38%, respectively; Bikker *et al.* (1994) reported a value of 74% for lysine; and Adeola (1995) estimated efficiency values of 72% and 60% for lysine and methionine, respectively. Using N balance in pigs has resulted in a range of efficiency estimates between 66% and 91% for a variety of indispensable amino acids (Heger *et al.* 2002, 2003). Methods using graded doses of amino acids in broiler chickens provided estimates of amino acid retention efficiency values of 82%, 80%, 73% and 61% for threonine (Edwards *et al.* 1997), lysine (Baker, 1991), valine (Baker *et al.* 1996) and isoleucine (Baker, 1991), respectively.

A 31% efficiency of retention of methionine in the current study on ducks together with the 49% estimate for valine from Timmler & Rodehutsord (2003) on ducks and the 21% estimate for tryptophan from Adeola (1998) point to the conclusion that the efficiency, above maintenance level, of amino acid retention is different for individual amino acids in ducks. A retention of dietary methionine with an efficiency of 31% in the current study indicates that more than 50% of the digested and absorbed methionine was used for purposes other than the synthesis of retained protein. As discussed by Shoveller *et al.* (2005), three important metabolic functions of methionine are: protein synthesis; transmethylation to form a primary methyl donor, *S*-adenosylmethionine, which is important in a variety of metabolic reactions; and transsulfuration to cysteine, which in turn is catabolized to taurine or incorporated into glutathione. Methionine metabolism to these putative derivatives, together with its oxidation during the normal physiological process of growth, accounts in major part for the low efficiency of methionine retention.

The observation that the methionine, cysteine, arginine and threonine concentrations of body protein increased in response to supplemental dietary methionine concentration is in agreement with previous work in which there were increases in the

concentration of a variety of amino acids in body protein from pigs and broiler chickens with increasing concentrations of dietary amino acids (Heger & Frydrych, 1985; Chung & Baker, 1992; Bikker *et al.* 1994; Adeola, 1995, 1998; Edwards *et al.* 1997). Furthermore, the amino acid intake-related changes in body amino acid concentration noted in the current study are consistent with the expected increase in proportion of contractile proteins relative to collagen protein. As the intake of a limiting amino acid increases, there is a larger increase in the concentration of indispensable amino acid in body protein owing to the larger proportion of contractile proteins relative to collagen protein (Bikker *et al.* 1994). The observed changes in concentration of some amino acids in body protein are indicative of the dependence of body amino acid concentration on nutrient intake. It is noteworthy that the accretion of 15 mg methionine/g body protein accretion in ducks between 22 and 42 d post-hatching fed 2.4–3.9 g digestible methionine/kg diet is remarkably similar to the 16 mg methionine/g protein gain in ducks between 1 and 22 d post-hatching fed 7.1–12.7 g valine/kg diet (Timmler & Rodehutsord, 2003).

In summary, for ducks from 22 to 42 d post-hatching, there was a constant utilisation of methionine over the range of intake 2.4–3.9 g digestible methionine/kg diet, with an efficiency of 31%, and in each gram of protein accretion, there was a retention of 15 mg methionine.

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