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INFLUENCE OF PROTEIN SUPPLY ON THREONINE EFFICIENCY AND THREONINE CATABOLISM IN HEPATIC MITOCHONDRIA OF CHICKS AND RATS*

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Abstract

This research investigated the relationship between efficiency of threonine (Thr) utilization and Thr oxidation in hepatic mitochondria in chicks and rats fed with graded levels (5.5–33.0% CP for chicks, 6–24% CP for rats) of protein. Calculation of efficiency of Thr utilization was based on N-balance data and an exponential N-utilization model, and Thr dehydrogenase (TDG) activity was determined. According to the results, no significant effect on TDG activity was observed in the liver of chicks who received diets containing from 5.5 up to 16.5% CP. However, significantly elevated TDG activities were observed, despite limited supply of Thr in protein, with diets containing from 22.0 up to 33.0% CP. At the levels of CP content from 5.5 up to 27.5%, no significant change in efficiencies of Thr utilization was observed. However, a significant decrease in efficiency was observed with diets containing from 27.5 to 33.0% CP. In chicks, the relationship between oxidation of Thr and Thr efficiency was observed with graded CP levels. In addition, elevated TDG activities in rat liver were observed with diets containing from 6.0 to 12.0% CP. At the levels of CP content from 6.0 up to 24.0%, no significant effect on efficiency of Thr utilization was observed in rats. In addition, no relationship was observed between Thr oxidation and efficiency of Thr utilization with graded CP levels in rats. Taken together, reactions for TDG appear to be animal species-dependent.

Key words: crude protein, N-utilization model, threonine dehydrogenase, threonine efficiency

Proteins are the major functional and structural components in the bodies of animals. They are composed of many amino acid subunits joined together through pep-

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tide bonds. There is a greater diversity of chemical composition in proteins (Berthold et al., 2005). Proteins in various animals have biological specificity. In the form of feathers, skin, cartilage, and muscles, proteins hold together and provide structure to the body of an organism (NRC, 1994). In addition, amino acids are the building blocks of proteins. The 22 amino acids, essential and nonessential, affect a broad range of physical processes (Reeds, 2000; Han and Thacker, 2011; Takahashi et al., 2011). Physiological concentrations of amino acids are required for biological functions in the bodies of animals. Some amino acids are essential for animals that cannot synthesize these essential amino acids in the body; therefore, they must be provided in the diet using synthetic amino acids. In addition, balance among the essential amino acids is of great importance (Vieira and Angel, 2012), because reduction of dietary protein content can only be effective when requirements for the essential amino acids are satisfied (Kerr and Kidd, 1999; Taghinejad-Roudbaneh et al., 2013). However, as the CP content is reduced, performance decreases, even when the requirements for all amino acids are met (Bregendahl et al., 2002; Si et al., 2004). Thus, the CP content is of importance in improving growth performance (Jiang et al., 2005; Waldroup et al., 2005). In addition, recent studies have demonstrated that amino acids are cell signaling factors as well as modulators of the protein phosphorylation cascade (Rhoads et al., 2008; Suryawan et al., 2008; Wu, 2009).

Oxidation of Thr results in irreversible loss of Thr and increases the metabolic need for this amino acid. Metabolic factors regulating the oxidation of Thr are of great importance in order to optimize Thr supplementation and to satisfy the physiological need for Thr (Samadi and Liebert, 2007; van der Sluis et al., 2009; Levesque et al., 2011; Rezaeipour et al., 2012). Thr is an indispensable amino acid for birds and mammals (Baylan et al., 2006; Ayasan et al., 2009; Canogullari et al., 2009; Ayasan and Okan, 2010). Oxidation of Thr occurs in the liver, mainly via two major pathways based on the enzymes Thr dehydratase (TDH, EC 4.2.1.16) and Thr dehydrogenase (TDG, EC 1.1.1.103) (Davis and Austic, 1994). TDH reacts as a cytosolic enzyme and yields 2-ketobutyric acid and NH_4^+. In addition, TDG is a mitochondrial enzyme producing Gly and aminoacetone (Davis and Austic, 1997; Yuan et al., 2000; Yuan and Austic, 2001; Ishikawa et al., 2007; Sartori et al., 2008; Lee et al., 2011, 2014). Therefore, oxidation of Thr via TDG can be a major pathway for the metabolic need for Gly (Le Floc'h et al., 1996).

The purpose of the current study was to investigate the effects of diets supplemented with graded levels of protein on efficiency of Thr utilization and TDG activity as an indicator for catabolic rate of Thr in chicks and rats.

Material and methods

Material

L-Thr and nicotinamide adenine dinucleotide (NAD^+) were purchased from Merck (Darmstadt, Germany) and coenzyme A (CoA), L-Lys·HCl, DL-Met, L-Ile, L-Phe, and L-Trp were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and experiments

Experiment 1

Day-old male chicks (Cobb 500®) were prepared for the experiments and fed a commercial starter diet up to the beginning of the trial period. A total of 36 chicks were used in the N-balance study according to age period (15 to 25 days). For the N-balance study, 36 birds were randomly allocated to six diets with a graded supply of protein (Table 1, replicate number: 6). Chicks were individually housed in metabolism cages with wire floors equipped with individual feeding and self-drinking systems. Room temperature during rearing started at 32°C for optimal growth in the first week after hatching and was decreased by 2°C per week; lighting was provided 24 hours per day. Initial average body weights within the age period were 503±21 g. The N-balance study within the age period used six chicks per diet. The individual experimental period was divided into an adaptation period (five days) and a consecutive excreta collection period (five days). This experiment was conducted using the procedures reported by Samadi and Liebert (2006). After completion of the N-balance study, the chicks were sacrificed for sampling of individual livers.

Table 1. Composition of diets for chicks (g/kg diet)

Ingredients	5.5% CP	11.0% CP	16.5% CP	22.0% CP	27.5% CP	33.0% CP
High-protein soybean meal (44% CP)	112.00	224.00	336.00	448.00	560.00	672.00
Soybean oil	15.00	15.00	15.00	15.00	115.00	150.00
Potato starch	802.68	695.59	588.09	480.49	276.48	130.76
Cellulose powder	18.00	14.50	11.00	7.50	0.40	0
Premix[1]	10.00	10.00	10.00	10.00	10.00	10.00
Monocalcium phosphate	28.51	25.00	21.50	18.00	14.50	11.00
$CaCO_3$	10.30	11.10	11.90	12.70	13.50	14.30
NaCl	0.90	0.90	0.80	0.70	0.60	0.50
MgO	0.70	0.10	0	0	0	0
L-Lys·HCl (99%)	0.45	0.90	1.34	1.79	2.24	2.70
DL-Met (99%)	1.46	2.91	4.37	5.82	7.28	8.74
Analyzed composition						
CP (%)	5.9	11.1	16.4	22.2	27.6	33.1
$AMEN_2$ (MJ/ kg)	12.95	12.95	12.95	12.95	12.95	12.95
Lys	3.84	7.70	11.58	15.48	19.19	23.01
Met + Cys	3.02	6.05	9.11	12.17	15.09	18.09
Thr	2.14	4.30	6.46	8.64	10.70	12.83
Trp	0.68	1.36	2.05	2.74	3.40	4.08
Arg	4.07	8.17	12.28	16.42	20.35	24.39
Gly	2.34	4.70	7.07	9.45	11.71	14.03
Gly+Ser	5.06	10.16	15.28	20.42	25.31	30.33
Ca	10.8	10.8	10.8	10.8	10.8	10.8
P	7.0	7.0	7.0	7.0	7.0	7.0

[1]Main ingredients per kg of premix (Co. Vilomix, Germany)**:** 175.0 g calcium, 80.0 g sodium, 1,200,000 IU vitamin A, 300,000 IU vitamin D_3, 3,000 mg vitamin E, 200 mg vitamin B_1, 480 mg vitamin B_2, 360 mg vitamin B_6, 1,500 mg vitamin B_{12}, 300 mg vitamin K_3, 2,700 mg niacin, 900 mg calcium-pantothenate, 90 mg folic acid, 5,000 mg biotin, 80.0 g choline chloride, 12.0 g manganese, 8.0 g zinc, 5.0 g iron, 3.0 g copper, 120 mg iodine, 55 mg cobalt, 42 mg selenium, 10.0 g BHT, 12.5 g monensine-Na.

[2]Calculated based on World's Poultry Science Association (1984) guidelines. Amino acid ratio: Lys [1] : Met+Cys [0.79] : Thr [0.56] : Trp [0.18] : Arg [1.06] : Gly [0.61] : Gly+Ser [1.31]. Threonine was adjusted as a limiting amino acid.

Experiment 2

This experiment was conducted as an N-balance study (five-day adaption period, five-day preperiod, and six-day collection period) with a total of 30 male albino rats (Wistar, average body weight at the beginning of the preperiod 118±5.8 g) housed in metabolic cages under standardized conditions (22°C room temperature, 12 h light/dark cycle). For the N-balance study, 24 rats were randomly allocated to four diets with a graded supply of protein (Table 2, replicate number: 6). Individual liver samples were taken after completion of the balance study.

Table 2. Composition of diets for rats (g/kg diet)

Ingredients	6% CP	12% CP	18% CP	24% CP
Casein	65.00	131.00	196.00	261.00
Wheat starch	650.76	567.80	475.90	384.20
Sucrose	100.00	100.00	100.00	100.00
Cellulose	50.00	50.00	50.00	50.00
Soybean oil	50.00	60.00	80.00	100.00
Mineral mix[1]	60.00	60.00	60.00	60.00
Vitamin mix[2]	20.00	20.00	20.00	20.00
DL-Met (99%)	0.80	4.30	7.80	11.20
L-Ile (99%)	0.34	0.70	1.10	1.40
L-Phe (98%)	2.70	5.40	8.00	10.70
L-Trp (99%)	0.40	0.80	1.20	1.50
Analyzed composition:				
CP (%)	6.2	12.1	18.2	24.1
ME (MJ/ kg)	14.09	14.09	14.09	14.09
Lys	5.08	10.23	15.31	20.38
Met + Cys	5.44	10.95	16.38	21.81
Thr	2.50	5.03	7.53	10.02
Trp	1.12	2.25	3.37	4.48
Ile	3.4	6.85	10.26	13.66
Phe	5.64	11.36	17.0	22.62
Ca	5.0	5.0	5.0	5.0
P	3.0	3.0	3.0	3.0

[1]Ingredients of mineral mix (company Altromin, Germany) per kg: 146,068 mg calcium, 97,355 mg phosphorus, 1,734 mg manganese, 388 mg zinc, 2,931 mg iron, 85 mg copper, 6.6 mg iodine, 2.1 mg cobalt, 3.8 mg selenium, 8,783 mg magnesium, 39,229 mg sodium, 116,487 mg potassium, 10,536 mg sulphur, 63,510 mg chlorine, 3.3 mg molybdenum, 70 mg f1uorine, 0.07 mg a1uminium.

[2]Ingredients of vitamin mix (company Altromin, Germany) per kg : 750,000 IU vitamin A, 25,000 IU vitamin D_3, 7,500 mg vitamin E, 1,000 mg vitamin B_1, 1,000 mg vitamin B_2, 750 mg vitamin B_6, 1.5 mg vitamin B_{12}, 500 mg vitamin K_3, 2,500 mg niacin, 2,500 mg pantothenic acid, 500 mg folic acid, 10 mg biotin, 50,000 mg choline-chloride, 5,000 mg *p*-aminobenzoic acid, 5,000 mg inositol, 1,000 mg vitamin C, 37,100 mg sulphur, 1.3 mg aluminium. Amino acid ratio: Lys [1] : Met+Cys [1.07] : Thr [0.49] : Trp [0.22] : Ile [0.67] : Phe [1.11]. Threonine was adjusted as a limiting amino acid.

Both N-balance studies should provide experimental data regarding the efficiency of Thr utilization in the graded levels of protein supplementation. All animal procedures and handling were conducted in accordance with animal welfare legislation and were approved by the ethics committee (Protocol number: PNU 3718) of Pusan National University (Busan, Republic of Korea).

Diets

Before the start of the experimental period, all chicks and rats received commercial pelleted standard diets and water supplied by self-drinking systems *ad libitum*. The chick diets contained a mixture of high-protein soybean meal (crude protein source) (Table 1). Experimental diets for rats (Table 2) were based on casein as a crude protein source. Diets for chicks were supplied to meet the recommendations of the NRC (1994), except for Thr. The NRC (1994) recommendations for chick are 0.74% Thr (20% CP diet) (three to six weeks of age). Based on these data, six levels of CP (5.5–33.0%) were examined (Table 1). The diets for rats were formulated to meet the recommendations of the NRC (1995), except for Thr, in order to perform evaluations of Thr efficiency. The NRC (1995) recommendations for rat are 0.62% Thr (17% CP diet) (NRC, 1978; 12% CP). Based on these data, four levels of CP (6.0–24.0%) were examined (Table 2). Diets for chicks were supplemented with crystalline amino acids (L-Lys·HCl, DL-Met) in order to maintain Thr as the first limiting amino acid in all diets, according to the recommendations of the NRC (1994). Consequently, the dietary amino acid patterns were unchanged in chicks, independent of dietary protein level. Potato starch replaced the protein source in order to achieve a graded dietary protein supply with a constant amino acid ratio (Table 1), indicating Thr as the created limiting amino acid. In addition, in order to maintain Thr as the first limiting amino acid in all diets for rats, supplementation with crystalline amino acids (DL-Met, L-Ile, L-Phe, and L-Trp) was provided according to the recommendations of the NRC (1995). Subsequently, the dietary amino acid patterns were unchanged in rats, independent of dietary protein level. Wheat starch replaced the protein source in order to achieve a graded dietary protein supply with a constant amino acid ratio (Table 2), indicating Thr as the limiting amino acid.

Preparation of liver extracts

The individual livers of six animals per treatment were collected. After preparation, the livers were immediately frozen in liquid nitrogen and kept at –80°C before homogenization in ice-cold 0.25 M sucrose solution. The liver mitochondria fraction was isolated from the homogenate using the method of Schneider and Hogeboom (1950). Sediments of mitochondria fraction were resuspended in a buffer solution (pH 7.4, 10 mmol/L Tris-HCl, 10 mmol/L KH_2PO_4, 110 mmol/L $KHCO_3$ and 5 mmol/L $MgCl_2 \times 6H_2O$).

Threonine dehydrogenase activity assay

The mitochondrial solution (0.5 ml) was incubated in a metabolic shaker bath for 30 min (0 min for blanks) at 37°C in stoppered test tubes for culture with 2 ml of a modified medium (pH 7.4, 10 mmol/L Tris-HCl, 10 mmol/L KH_2PO_4, 110 mmol/L $KHCO_3$, 5 mmol/L $MgCl_2 \times 6H_2O$, 25 mmol/L L- Thr, 2.5 mmol/L NAD^+, and 1 mmol/L CoA) using the method described by Davis and Austic (1997). Following incubation, the reaction was terminated with 1 ml of trichloracetic acid (0.92 mol/L). The precipitated protein was removed by centrifugation at 4°C and the supernatant was used for the assay of aminoacetone and Gly. Aminoacetone was determined using the rapid colorimetric method and Gly was detected by column chromatography

in an automatic amino acid analyzer LC 3000 (Biotronik). The Biuret-assay with bovine serum albumin standards was used for measurement of protein concentration.

N-utilization model and evaluation of efficiency of Thr utilization

Calculation of the efficiency of Thr was based on N-balance data and an exponential N-utilization model (Samadi and Liebert, 2006), which was adapted to describe the relationship between concentration of the limiting amino acid (LAA) (c) and protein quality (b). The slope of the linear function (bc^{-1}) is used directly as an indicator for the efficiency of the utilization of the limiting amino acid in the diet (Liebert, 1995; Rimbach and Liebert, 2000; Thong and Liebert, 2004).

Principles of an exponential N-utilization model (Liebert, 1995; Thong and Liebert, 2004; Samadi and Liebert, 2006) were applied for analysis of the N-balance data:

$$NR + NR_{max}T(1 - e^{-b \cdot NI}) \tag{1}$$

where:

NR = daily N retention (mg/$BW_{kg}^{0.67}$),

$NR_{max}T$ = theoretical maximum for daily N retention (mg/$BW_{kg}^{0.67}$),

NI = daily N intake (mg/$BW_{kg}^{0.67}$),

b = model parameter for the slope of the function between NI and NR, depending on the dietary protein quality,

e = basic number of the natural logarithm.

Modeling of amino acid requirements may run for graded dietary amino acid efficiency within the variation of observed efficiency of amino acid utilization. Due to logarithmization and transformation of Equation (1), Equation (2) was applied for establishment of a model parameter from N-balance studies with a graded protein supply (Samadi and Liebert, 2006):

$$b = ln[NR_{max}T - ln(NR_{max}T - NR)]/NI \tag{2}$$

where:

b = model parameter of dietary protein quality,

ln = natural logarithm,

$NR_{max}T$ = theoretical maximum for daily N retention (mg/$BW_{kg}^{0.67}$),

NR = daily N retention (mg/$BW_{kg}^{0.67}$),

NI = daily N intake (mg/$BW_{kg}^{0.67}$).

In general, the concentration of LAA in the feed protein and the resulting dietary protein quality are linearly correlated. The slope of the linear function (quotient bc^{-1}) indicates the efficiency of utilization of LAA in the diet (Liebert, 1995). In addition, parameter (bc^{-1}) summarizes the efficiency within the processes of digestion and absorption and postabsorptive utilization. Calculation of the efficiency of Thr utiliza-

tion (bc^{-1}) is based on the analyzed Thr concentration [bc^{-1} = slope between b and c; b = model parameter of dietary protein quality; c = concentration of LAA in the feed protein (g/100 g of CP)].

Statistical analysis

Data were subjected to ANOVA (analysis of variance) using SPSS Base 14.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, IL 60606-6307, USA, 2005) and significant differences between mean values were determined using Tukey's test at $P<0.05$. Data are expressed as mean ± SD.

Results

Experiment 1

No significant effect on TDG activity was observed in the liver of chicks who received diets containing from 5.5 up to 16.5% CP. However, significantly elevated TDG activities were observed, despite limited supply of Thr in protein, with diets containing from 22.0 up to 33.0% CP (Table 3, $P<0.05$). At the levels of CP content from 5.5 up to 27.5%, no significant change was observed in the efficiencies of Thr utilization. However, a significant decrease in efficiency was observed with diets containing from 27.5 to 33.0% CP (Table 3, $P<0.05$). A summary of data from N-balance experiments with chicks is shown in Table 4.

Table 3. Effects of protein levels of the diets on liver TDG activity and efficiency of Thr utilization in chicks

Diet	CP-content	Thr efficiency (bc^{-1})*	TDG-activity (nmol/ 30 min/ mg protein)		
			Total activity	Aminoacetone	Glycine
A	5.5%	115 a±3	0.95 a±0.11	0.18 a±0.11	0.77 a±0.06
B	11.0%	115 a±3	1.25 a±0.22	0.54 a±0.20	0.71 a±0.11
C	16.5%	126 a±4	2.48 a±0.27	1.49 a±0.25	0.98 a±0.20
D	22.0%	124 a±5	12.79 b±1.68	8.91 b±0.87	3.88 a±1.95
E	27.5%	123 a±4	24.40 c±6.68	11.03 b±3.29	13.37 b±4.42
F	33.0%	97 b±3	95.90 d±7.44	28.70 c±1.40	67.20 c±6.24

Values indicate mean ± SD (n = 6). *Calculated from N-balance data, exponential N-utilization model. Different letters within a row indicate significant differences ($P<0.05$; Tukey's test).

Table 4. Summarized data from N-balance experiments (n = 6) with chicks (genotype Cobb 500) fed with a crude protein level according to age (days 20–25)

Item	Diet					
	A (5.5% CP	B 11.0% CP	C 16.5% CP	D 22.0% CP	E 27.5% CP	F 33.0% CP
BWG (g)	30 a±2.5	215 b±14	323 c±17	327 c±15	329 c±18	321c±25
Feed intake (g)	307.2 a±7.9	463.9 b±9.3	477.0 b±12.2	464.0 b±6.9	451.1 b±8.5	415.6 b±9.7
FCR (g/g)	10.2 a±0.15	2.16 b±0.07	1.48 c±0.06	1.42 c±0.05	1.37 c ±0.04	1.30 c±0.06
N retention[1]	771 a±66	1,617 b±136	2,093c±184	2,193 c±365	2,394 c±235	2,413c±67

Values indicate mean ± SD (n = 6). [1]$mg/BW_{kg}^{0.67}$ per day. Different letters within a row indicate significant differences ($P<0.05$; Tukey's test). BWG: body weight gain, FCR: feed conversion ratio.

Experiment 2

Elevated TDG activities in rat liver were observed with diets containing from 6.0 to 12.0% CP ($P<0.05$). However, significantly reduced TDG activities were observed with diets containing from 12.0 to 18.0% CP ($P<0.05$). In addition, no significant effect on TDG activities was observed with diets containing from 18.0 to 24.0% CP. This effect resulted from an increase of aminoacetone (Table 5). At the levels of CP content from 6.0 up to 24.0%, no significant effect on the efficiencies of Thr utilization was observed (Table 5). A summary of data from N-balance experiments with rats is shown in Table 6.

Table 5. Effects of protein levels of the diet on liver TDG activity and efficiency of Thr utilization in growing rats

Diet	CP- content	Thr efficiency (bc^{-1})*	TDG-activity (nmol/30 min/mg protein)		
			Total activity	Aminoacetone	Glycine
A	6%	340±14	12.11 ab±2.89	10.51 ab±2.60	1.60±0.44
B	12%	389±24	15.93 b±4.21	14.25 b±3.80	1.68±0.46
C	18%	383±27	10.23 a±2.67	8.94 a±2.59	1.29±0.64
D	24%	382±26	12.56 ab±1.49	10.93 ab±1.14	1.63±0.56

Values indicate mean ± SD (n = 6). *Calculated from N-balance data, exponential N-utilization model. Different letters within a row indicate significant differences ($P<0.05$; Tukey's test).

Table 6. Summarized data from N-balance experiments (n = 6) with rats fed with a crude protein level according to age (days 38–44)

Item	Diet			
	A 6% CP	B 12% CP	C 18% CP	D 24% CP
BWG (g)	14 a±1.5	24 b±3.1	26 b±2.7	28 b±3.1
Feed intake (g)	71.8±0.2	71.5±0.2	71.9±0.1	71.5±0.5
FCR (g/g)	5.12 a±0.56	2.97 b±0.31	2.77 b±0.31	2.55 b±0.29
N retention[1]	418 a±22	714 b±29	756 bc ±63	811 c±70

Values indicate mean ± SD (n = 6). [1]$mg/BW_{kg}^{0.67}$ per day. Different letters within a row indicate significant differences ($P<0.05$; Tukey's test). BWG: body weight gain, FCR: feed conversion ratio.

Discussion

The TDG activities of the liver in chicks were elevated by increasing the CP content in the diets. These results indicate that administration of a high protein diet leads to an increase in hepatic TDG activity. These findings are in general agreement with results reported by Yuan and Austic (2001) indicating that the activity of TDG in liver mitochondria was significantly higher in the 32% CP diet than in the 23% CP diet, and the activity in the 27% CP diet was intermediate. In particular, we demonstrated that despite existing limitation of Thr, measured relatively to the amino acids lysine, methionine/cystine, tryptophan, and arginine, catabolism of Thr in diets from 22.0 up to 33.0% CP was significantly increased. These findings indicate that the catabolism of Thr probably follows a general increase in the non-specific catabolism

rate of other amino acids. This observation supports the findings reported by Davis and Austic (1997) indicating that the cellular concentration of other amino acids in addition to Thr can influence TDG activity in the liver of chicks. Very low levels of TDG activity were observed with diets containing CP and Thr below the required levels. These results are in general agreement with results reported by Benevenga et al. (1993) indicating that a minimal oxidation of amino acids occurs in diets that are below the requirement for high growth. Thus, our results suggest that catabolism of Thr in the liver of chicks is stimulated by the protein concentration in the diet.

An increase in TDG activity between 27.5 and 33.0% CP showed an association with significantly lower efficiency of Thr utilization. A significant decrease of efficiency of Thr utilization in the 33.0% CP diet indicated a close association with sharp increase of Thr catabolism. These results are in general agreement with findings reported by Lee et al. (2011) in chicks showing that TDG activity in the liver was elevated by the diet containing 22.5% CP (0.60% Thr) and the efficiency of Thr utilization was decreased. However, it is also clear that the changes of TDG activity with low levels of CP had no significant effects on efficiency of Thr utilization. These results underline the limited quantitative meaning of TDG activity measured *in vitro*. Therefore, our findings suggest that efficiency of Thr utilization is reduced by enhanced degradation of Thr through TDG in chicks.

TDG activities in rat liver were not enhanced by an increase in the content of CP in the diets. Administration of a diet supplemented with a high level of CP did not lead to a sharp increase in hepatic TDG activity. This result is in general agreement with results reported by Bird and Nunn (1983). According to the recommendations of the NRC (1978, 1995), the protein requirement for maximum growth in rats was in the range of 12.0% CP when highly digestible protein was used with a balanced amino acid pattern. Mercer et al. (1989) reported that 19% casein (approximately 17% CP) was necessary in the diet in order to reach 95% of the maximum capacity for growth. Despite a low limitation of Thr in CP, but in diets with total requirements covering CP- and Thr-supply (18.0% CP and 0.75% Thr) and in diets with requirements exceeding supply (24.0% CP and 1.0% Thr), TDG activity was not significantly increased. This finding indicates that TDG activity has a weak dependence on the CP- and Thr-content in the range of optimal CP- and Thr-requirements. In the optimal range (18.0% CP and 0.75% Thr), the TDG activity was low. This finding is in general agreement with results reported by Rees et al. (2006) indicating that the activity of TDG in the liver was unaffected by the rats fed the diet containing 18% CP. Catabolism of Thr showed a significant decrease when the CP content was increased from 12.0 to 18.0%. The ratio of the total amount of Gly and serine in diets containing 18.0% and 12.0% CP was 1.5:1. Namely, the 18.0% CP diet contained a 1.5-fold higher amount of Gly and serine. Thr in the diet containing 18.0% CP might be degraded to a small extent to Gly through TDG, because sufficient amino acids at the level of the protein are available to ensure adequate growth. According to the results reported by Ballèvre et al. (1990), who conducted *in vivo* studies in pigs, approximately 80% of Thr was degraded to Gly. In addition, based on *in vitro* studies with rat hepatic mitochondria, Bird et al. (1984) reported that at least 65% of Thr was converted to Gly and acetyl-CoA with an adequate supply of Thr. These results

indicate that at least 60% of Thr is converted to Gly. Here, differences were observed between *in vivo* and *in vitro* measurements. Nevertheless, according to reports from Bird et al. (1984) and Ballèvre et al. (1990), it could be concluded that approximately 60–80% of Thr can be degraded to Gly. Thus, it is considered that *de novo* synthesis of Gly from Thr through TDG is an important metabolic pathway to cover greater need for Gly.

In rats, the increase in TDG activity between 6.0 and 12.0% CP was not associated with significant changes of efficiency of Thr utilization. In the graded CP levels (6.0–24.0% CP), no relationship was observed between catabolism of Thr and efficiency of Thr utilization. Thus, there was no evident association for the catabolism of Thr through TDG and efficiency of Thr utilization at graded CP levels. In particular, despite the oversupply of CP, catabolism of Thr was not intensively modulated through TDG in rats. These findings differed outstandingly from the observations in chicks. Therefore, conduct of further studies will be required in order to fully understand the relationship between catabolism of Thr and efficiency of Thr utilization in rats.

In conclusion, our findings suggest that catabolism of Thr in the liver of chicks is modulated by the protein concentration in the diet and that efficiency of Thr utilization is reduced by enhanced degradation of Thr through TDG. In addition, an increase in TDG activity could be a reflection of the higher requirements for Gly for uric acid formation in chicks. In rats, feeding with a graded protein supply did not result in additional stimulation of Thr catabolism through TDG over a wide range of CP content in the diets. In addition, no relationship was observed between catabolism of Thr and efficiency of Thr utilization in the graded CP levels in rats. Taken together, it appears that reactions for TDG are animal species-dependent.

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