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Effects of Dietary Protein and Threonine Supply on *In vitro* Liver Threonine Dehydrogenase Activity and Threonine Efficiency in Rat and Chicken

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ABSTRACT : This study was conducted to assess the relation between threonine (Thr) oxidation rate and threonine efficiency on rat and chicken fed with graded levels of protein and threonine. The increase in threonine content from 0.28 to 0.72% in a diet containing 12.0% crude protein (CP) caused a gradual increase in threonine dehydrogenase (TDG) activity in rat liver. Similar, but more pronounced results were observed after 18.0% CP in the diet. Both protein levels in combination with the highest level of threonine supplementation increased liver TDG activity significantly, indicating enhanced threonine catabolism. Parameters of efficiency of threonine utilization calculated from parallel nitrogen balance studies decreased significantly and indicated threonine oversupply after a maximum of threonine supplementation. At the lower levels of threonine addition the efficiency of threonine utilization was not significantly changed. In the chicken liver up to 0.60% true digestible threonine (dThr) in the 18.5% CP diet produced no effect on the TDG activity. However, TDG activity in the liver was elevated by the diet containing 22.5% CP (0.60% dThr) and the efficiency of threonine utilization decreased, indicating the end of threonine limiting range. In conclusion, the *in vitro* TDG activity in the liver of rat and growing chicken has an indicator function for the dietary supply of threonine. (**Key Words :** Threonine Oxidation, Threonine Efficiency, Threonine Dehydrogenase, Liver of Rat and Chicken)

INTRODUCTION

Threonine is an indispensable amino acid for mammals and birds. Threonine degradation means an irreversible loss of threonine and increases the metabolic requirement for this amino acid. A detailed knowledge of the metabolic factors controlling threonine metabolism (Rees et al., 2006; van der Sluis et al., 2009) is needed in order to increase the understanding of how to optimize threonine supply and to meet the physiological threonine requirement (Le Floc'h et al., 1994; Samadi and Liebert, 2007; Levesque et al., 2011).

Threonine catabolism takes place in the liver, mainly through two major pathways (Bird and Nunn, 1983) based on the enzymes threonine dehydratase (TDH, EC 4.2.1.16) and threonine dehydrogenase (TDG, EC 1.1.1.103). The enzyme TDH acts as a cytosolic enzyme and yields 2-ketobutyric acid and NH_4^+ (Inoue and Pitot, 1970; Kang-Lee and Harper, 1978). The enzyme TDG (Lee and Liebert, 2001; Ishikawa et al., 2007) is a mitochondrial enzyme (Dale, 1978), yielding glycine and aminoacetone (Aoyama and Motokawa, 1981; Sartori et al., 2008). The TDG is located in the mitochondrial matrix and forms a soluble complex with 2-amino-3-oxobutyrate CoA ligase (glycine acetyltransferase; EC 2.3.1.29), which catalyzes the transformation of 2-amino-3-oxobutyrate to acetyl-CoA and glycine (McGilvray and Morris, 1969; Bird and Nunn, 1979). The de novo glycine synthesis from threonine might partly cover the metabolic requirement for glycine through the TDG-pathway (Le Floc'h et al., 1994). Glycine can be defined as an essential amino acid for the growing chicken (Baker et al., 1968) and indispensable for the synthesis of purine bases including the synthesis of uric acid. Aminoacetone is formed nonenzymatically by rat (Green and Elliot, 1964) and human (Mauzerall and Granick, 1956).

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TDG is an important enzyme for threonine degradation. This metabolic pathway is responsible for 87% of the total threonine catabolism in the liver of rats (Bird and Nunn, 1983) and for about 80% of catabolism in the liver of pigs (Ballèvre et al., 1990).

The investigation was conducted to evaluate *in vitro* TDG-activity as an indicator for threonine degradation rate depending on dietary protein and threonine supply in rat and growing chicken. Furthermore, by comparing the results with data from threonine efficiency studies, the physiological significance of *in vitro* determinations of this enzyme activity in liver tissue should be evaluated.

MATERIALS AND METHODS

Material

All chemicals obtained from commercial suppliers were of analytical reagent grade or the highest purity available. L-Threonine (Thr) and NAD were obtained from Merck (Germany), and CoA was obtained from Sigma (Germany).

Animals and experiments

The study was divided in 2 experiments.

Experiment 1 : This experiment was conducted as N-balance study (5 days adaption period, 5 days preperiod and 6 days collecting period) with totally 36 male albino rats (Wistar, SPF; 157-167 g mean body weight, 28 day-old) housed in modified metabolic cages after Horszczaruk and Bock (1963) under standardized conditions (22°C room temperature, 12 h light/dark cycle). For the N-balance study, 36 rats were randomly allocated to 6 diets with a graded protein and threonine supply (Tables 4 and 6). Individual liver samples were taken after finishing the balance study.

Experiment 2 : This experiment was conducted as N-balance study with 24 male growing chickens (Cobb 500; 20 day-old) in metabolic cages (collecting period day 20-25). For the N-balance study, 24 chickens were randomly allocated to 4 diets with a graded protein and threonine supply (Tables 5 and 7). A parallel growth study (days 17-30) with totally 24 male growing chickens (Cobb 500; 17 day-old) was conducted for sampling of individual livers. 24 male chickens were randomly allocated to 4 diets with a graded protein and threonine supply (Tables 5 and 7) for the growth study. Room temperature during rearing started with 32°C for optimal growth in the first week after hatching and declined by 2°C per week, lighting was 24 hours per day.

Both N-balance studies should provide experimental data about the efficiency of threonine utilization in threonine limiting basal diets and furthermore about the effects of graded levels of threonine supplementation on protein deposition. All animal procedures and handlings were conducted in accordance with animal welfare legislations and were approved by the ethics committee of Goettingen University.

Diets

Before the start of the experimental period all animals received commercial pelleted standard diets and water by self drinking systems *ad libitum*. Experimental diets for rats (Table 4) were based on wheat gluten as protein source. The chicken diets contained a mixture of wheat and wheat gluten (Table 5). Diets were supplemented with amino acids to achieve an amino acid composition of the protein fraction according to ideal protein (Gahl et al., 1991; Baker and Han, 1994) with the exception of threonine as limiting amino acid. Crystalline threonine (L-Thr) was only supplemented to make diets with equal threonine supply but different protein concentration. The diets for rat were formulated to meet the recommendations of NRC (1995) except for threonine to apply evaluations of threonine efficiency. The NRC (1995) recommendations are 0.62% Thr (17% CP diet) for rat (NRC, 1978; 12% CP). Based on these data, two levels of CP (12.0 and 18.0%) and three levels of threonine (main levels; 0.42 and 0.72%) were examined. The diets for chicken were supplied to meet the recommendations of NRC (1994) except for threonine. The NRC (1994) recommendations are 0.74% Thr (20% CP diet) for chicken (3 to 6 week of age). Based on these data, two levels of CP (18.5 and 22.5%) and two levels of dThr (0.45 (0.50 and 0.52% Thr) and 0.60% (0.65 and 0.67% Thr)) were examined.

To achieve defined diets with equal threonine supply, diets for rat were formulated in threonine contents of 0.42 and 0.72% in a diet containing 12.0 and 18.0% CP. The diets containing 12.0% CP (0.42 and 0.72% Thr) were made by the addition of 1.45 and 4.45 g threonine to basal diet (Table 4), respectively. In Addition, the diet containing 18.0% CP (0.42% Thr) was supplied with only basal diet and the diet containing 18.0% CP (0.72% Thr) was made by the addition of 3.05 g threonine to basal diet. Diets for chicken were formulated in contents of 0.45 and 0.60% true digestible threonine (dThr) in a diet containing 18.5 and 22.5% CP (18.5% CP-0.45% dThr (0.50% Thr), 22.5% CP-0.45% dThr (0.52% Thr), 18.5% CP-0.60% dThr (0.65% Thr), 22.5% CP-0.60% dThr (0.67% Thr)).

Preparation of liver extracts

The individual livers of 6 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 $MgCl_2 \cdot 6H_2O$).

Threonine dehydrogenase 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 $MgCl_2 \cdot 6H_2O$, 25 mmol/L L-Threonine, 2.5 mmol/L NAD^+ and 1 mmol/L CoA) after Bird et al. (1984). After 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 glycine. Aminoacetone was determined by the method of Urata and Granick (1963) and glycine was detected by column chromatography in an automatic amino acid analyzer LC 3000 (Biotronik). The protein concentration was measured by Biuret-assay with bovine serum albumin standards.

N-utilization model and evaluation of threonine efficiency

The efficiency of threonine was calculated based on N-balance data and an exponential N-utilization model (Gebhardt, 1966), 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 directly used as an indicator for the efficiency of the utilization of the limiting amino acid in the diet (Liebert and Gebhardt, 1980; Liebert et al., 1987; Liebert, 1995; Rimbach and Liebert, 2000; Thong and Liebert, 2004).

Principles of an exponential N-utilization model (Gebhardt, 1966; Liebert, 1995; Thong and Liebert, 2004; Samadi and Liebert, 2006) were applied for analysis of the N-balance data.

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

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 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 to establish a model parameter from N-balance studies with graded protein supply (Samadi and Liebert, 2006).

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

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 ($mg/BW_{kg}^{0.67}$).

Generally, the concentration of the LAA in the feed protein and the resulting dietary protein quality are linearly correlated (Liebert and Gebhardt, 1980). The slope of the linear function (quotient bc^{-1}) indicates the efficiency of utilization of the LAA in the diet (Liebert, 1995). Furthermore, parameter (bc^{-1}) summarizes the efficiency within the processes digestion and absorption and postabsorptive utilization. Efficiency of Thr utilization (bc^{-1}) is calculated based on the analyzed Thr concentration (bc^{-1} = slope between b and c; b = model parameter of dietary protein quality; c = concentration of the LAA in the feed protein (g/100 g of CP)).

Statistical analyses

The data were subjected to ANOVA (Analysis of Variance) (SPSS, version 14) and significant differences between mean values were determined by Tukey's test at $p < 0.05$. The data are expressed as mean±SD.

RESULTS

Experiment 1

The results with laboratory rats (Table 6) show a gradual and partly significant increase of total TDG activity (Aminoacetone+Glycine) in rat livers corresponding to the increase of threonine supply from 0.28% (basal diet without threonine addition, A) to 0.72% (C) in the diets containing 12.0% CP. After application of diets containing 18.0% CP and 0.42% Thr (basal diet without threonine addition, D) up to 0.72% Thr (F) a similar tendency was observed but at slightly higher level. After the diets with the highest level of threonine supplementation (C resp. F) the TDG activity was significantly increased, indicating enhanced threonine catabolism. However, the TDG activity was almost exclusively modulated by aminoacetone accumulation. Parameters of threonine efficiency (Table 6) obviously showed a response to threonine oversupply following diets C and F. Consequently, the threonine efficiency was significantly decreased. The lower the value of threonine efficiency (bc^{-1}) is, the nearer the threonine level of the diet is to the optimal threonine concentration. In the range of threonine oversupply its value is lowered, too. Therefore, the threonine efficiency of diet C group was much lower than that of diet F group because the threonine level of diet C group was nearer than that of diet F group to the optimal threonine concentration or in the range of more oversupply of threonine. Summarized data of N-balance experiments

Table 1. Summarized data of N-balance experiments (n = 6) with rats (days 38-44)

Item \ Diet	A (12.0% CP-0.28% Thr)	B (12.0% CP-0.42% Thr)	C (12.0% CP-0.72% Thr)	D (18.0% CP-0.42% Thr)	E (18.0% CP-0.52% Thr)	F (18.0% CP-0.72% Thr)
Mean BW (g)	157±12	169±6	177±9	167±8	177±7	179±5
Feed (DM) intake (g/d)	13.7±0.6	14.0±0.1	13.9±0.3	14.0±0.4	14.0±0.2	13.8±0.5
N retention*	593±25	723±15	690±38	748±23	821±35	801±27

Values are mean±SD (n = 6). * mg/$BW_{kg}^{0.67}$ per day.

with rats are presented in Table 1.

Experiment 2

The results with growing chickens (Table 7) at a crude protein level of 18.5% demonstrate that the total TDG activity does not directly respond to the dietary supplementation of threonine. Otherwise, the addition of threonine to the diet with a higher crude protein level (22.5% CP) increased the TDG activity significantly. This effect resulted from an increase of aminoacetone and glycine concentration. We used two levels of dThr (0.60% dThr (level near to the optimal threonine concentration) and 0.45% dThr (low level not near to the optimal threonine concentration)). The efficiency of threonine utilization, concluded from N-balance studies, was significantly reduced after supplementation of threonine (B, D) at both protein levels, indicating the loss of the limiting range for threonine. The lower the value of threonine efficiency (bc^{-1}) is, the nearer the threonine level of the diet is to the optimal threonine concentration. Therefore, the threonine efficiencies of diet A and B groups were much lower than those of diet C and D groups because the threonine levels of diet A and B groups were nearer than those of diet C and D groups to the optimal threonine concentration. In addition, the threonine level of diet B group was nearer than that of diet D group to the optimal threonine concentration. Namely, the threonine level of diet B group was the nearest of all groups to the optimal threonine concentration. Summarized data of N-balance experiments and growth study with chickens are presented in Tables 2 and 3. In Addition, the relation between aminoacetone and glycine formation during *in vitro* incubation seems to be different between rat and growing chicken.

DISCUSSION

The threonine catabolism in rat livers (Table 6), concluded after *in vitro* incubation of liver tissue, increased significantly following graded dietary threonine supply (0.28 to 0.72% Thr) in 12.0% CP diets and 18.0% CP diets (0.42 to 0.72% Thr). These observations demonstrate that the TDG-activity responds to the threonine supply in the diet. The TDG-activities at similar total threonine levels in the diet (B and D resp. C and F) are not significantly different. However, after the diets with the highest level of threonine supplementation (C resp. F) the TDG activity was significantly increased indicating a stimulation of threonine catabolism (Rees et al., 2006; van der Sluis et al., 2009).

Table 2. Summarized data of N-balance experiments (n = 6) with chickens (genotype Cobb 500) (days 20-25)

Item \ Diet	A (18.5% CP-0.45% dThr*)	B (18.5% CP-0.60% dThr)	C (22.5% CP-0.45% dThr)	D (22.5% CP-0.60% dThr)
Mean BW (g)	703±59	714±39	722±50	782±28
Feed (DM) intake (g/d)	65.1±6.8	63.3±5.7	66.1±3.6	66.3±3.3
N retention**	1,410±96	1,500±85	1,643±55	1,820±39

Values are mean±SD (n = 6). * True digestible threonine (AminoDat 1.1, Degussa 1997). ** mg/$BW_{kg}^{0.67}$ per day.

Table 3. Summarized data of growth study (n = 6) with chickens (genotype Cobb 500) (days 17-30)

Item \ Diet	A (18.5% CP-0.45% dThr*)	B (18.5% CP-0.60% dThr)	C (22.5% CP-0.45% dThr)	D (22.5% CP-0.60% dThr)
Mean BW (g)	1,050±20	1,063±15	1,073±19	1,149±22
Feed (DM) intake (g/d)	96.8±5.0	99.2±3.8	99.4±3.4	101.6±3.7
BW gain (g/d)	61.9±2.6	64.4±2.7	65.6±1.9	74.7±3.0

Values are mean±SD (n = 6). * True digestible threonine (AminoDat 1.1, Degussa 1997).

Table 4. Composition of basal diets for laboratory rats (g/kg diet)

Ingredients	12% CP	18% CP
Wheat gluten	148.00	223.00
Sucrose	100.00	100.00
Cellulose	50.00	50.00
Soybean oil	55.00	50.00
Mineral mix**	60.00	60.00
Vitamin mix***	20.00	20.00
DL-methionine	1.60	3.80
L-threonine	0/1.45/4.45	0/1.05/3.05
L-isoleucine	0.76	1.20
L-leucine	2.58	3.90
L-lysine·HCl	5.44	8.23
L-phenylalanine	1.51	2.30
L-tryptophan	0.50	0.80
L-valine	1.25	1.90
Wheat starch	ad 1000	ad 1000
Amino acid ratios*	Lys(1):Thr(0.40)	Lys(1):Thr(0.40)
ME (MJ/ kg DM)	16.54	16.46

* Lys (1):Met/Cys (1.07):Trp (0.22):Ile (0.67):Phe (1.11):Gly (0.52):Gly+Ser (1.06) in basal diets.

** Main ingredients per kg of mineral mix (Co. Altromin, Germany): 146.06 g calcium, 97.35 g phosphorus, 116.49 g potassium, 39.23 g sodium, 63.51 g chloride, 8.78 g magnesium, 10.54 g sulphur, 1.73 g manganese, 388 mg zinc, 2.93 g iron, 85 mg copper, 6.6 mg iodine, 2.1 mg cobalt, 3.8 mg selenium, 3.3 mg molybdenum, 70 mg fluorine, 0.07 mg aluminium.

*** Main ingredients per kg of vitamin mix (Co. Altromin, Germany): 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-aminobenzoicacid, 5,000 mg inositol, 1,000 mg vitamin C.

Table 5. Composition of basal diets for growing chickens (g/kg diet)

Ingredients	18.50% CP	22.50% CP
Wheat	588.80	739.20
Wheat gluten	94.20	118.30
Wheat starch	213.30	30.10
Soybean oil	28.00	40.00
Premix*	10.00	10.00
Mono calcium phosphate	20.50	18.00
$CaCO_3$	12.00	13.00
NaCl	3.00	3.00
MgO	0.40	0
Celite	10.00	10.00
L-threonine	0.93	0
L-lysine·HCl	6.14	7.71
DL-methionine	1.49	1.87
L-tryptophan	0.24	0.30
L-arginine	2.83	3.62
L-isoleucine	0.97	1.20
L-glycine	7.20	3.70
Amino acids ratios**	Lys (1):Thr (0.53):Gly (1.30):Gly+Ser (2.02)	Lys (1):Thr (0.44):Gly (0.89):Gly+Ser (1.61)
ME_N (MJ/kg DM)	15.40	15.06

* 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, 1.5 mg vitamin B_{12}, 300 mg vitamin K_3, 5.0 g niacin, 900 mg calcium-pantothenate, 90 mg folic acid, 5 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.

** Lys (1):Met+Cys (0.75):Trp (0.19):Arg (1.05) in basal diets.

Table 6. Effects of protein and threonine levels of the diet on liver TDG activity and threonine efficiency in rats

Diet	CP-/Thr content	Mean liver weight (g)	TDG activity (nmol/ 30 min/mg protein)			Thr efficiency (bc^{-1})*
			Aminoacetone	Glycine	Total activity	
A	12.0% CP	9.3	7.70 [a]	0.86 [a]	8.56 [a]	502 [ad]
	0.28% Thr	±0.8	±1.77	±0.31	±1.73	±13
B	12.0% CP	9.7	9.96 [ac]	0.91 [ac]	10.87 [ac]	527 [ad]
	0.42% Thr	±0.5	±0.88	±0.26	±0.92	±20
C	12.0% CP	10.1	12.22 [bc]	0.77 [a]	12.99 [bc]	288 [b]
	0.72% Thr	±0.6	±3.39	±0.28	±3.37	±29
D	18.0% CP	10.5	10.59 [ac]	1.02 [ac]	11.60 [ac]	578 [ac]
	0.42% Thr	±0.7	±2.18	±0.21	±2.20	±45
E	18.0% CP	11.1	13.13 [bc]	0.60 [a]	13.74 [bc]	674 [c]
	0.52% Thr	±1.2	±2.61	±0.28	±2.68	±21
F	18.0% CP	10.3	15.16 [b]	1.42 [bc]	16.57 [b]	445[d]
	0.72% Thr	±1.0	±1.38	±0.46	±1.65	±51

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

These observations are in general agreement with results from Chu and Hegstedt (1976) and Kang-Lee and Harper (1978) for threonine but also for increasing dietary levels for other essential amino acids (Aguilar et al., 1974; Brookes et al., 1972; Kim et al., 1983). Furthermore, it was demonstrated by Bloxam (1975) that threonine oxidation was very low at low levels of dietary threonine supply. Otherwise, Yamashita and Ashida (1971) observed higher oxidation rates for threonine compared to lysine following an excessive dietary supply of these amino acids in rat diets.

The results of rat N-balance studies and conclusions about the efficiency of threonine utilization (Table 6) clearly indicate that threonine was only limiting amino acid in rat diets A, B and D, E, respectively. This observation is in agreement with earlier supplementation studies (Liebert and Gebhardt, 1979, 1980) and indicates the sensitivity of this parameter for the utilization of the limiting amino acid. Compared to the results of TDG activity a more clear decision relating to the limiting area of threonine is possible.

In the chicken liver (Table 7) an increase of dietary threonine supply (from 0.45 to 0.60% true digestible threonine) resulted in no significant effect on the TDG activity after diets containing 18.5% CP. This observation is supported by results of Davis and Austic (1997) in chicken, indicating that the hepatic TDG activity was predominantly affected by the protein level in the diet. However, after diets containing 22.5% CP at similar level of true digestible threonine (0.45%) the TDG activity was not significantly enhanced. A significant increase of TDG activity in the liver of growing chicken was only observed after elevation of the dietary threonine supply from 0.45 to 0.60% true digestible threonine in diets containing 22.5% CP. According to the results of Le Floc'h et al. (1994, 1996) in growing pigs, our results indicate that the effect on hepatic TDG activity was more pronounced depending on the threonine level in the diet. Obviously, after the low protein diet (18.5% CP) a moderate excess of threonine by supplementation of threonine was not significantly oxidized through threonine dehydrogenase pathway by chicken liver. A stimulation of degradation by threonine dehydratase pathway cannot be

Table 7. Effects of protein and threonine levels of the diet on liver TDG activity and threonine efficiency in growing chickens

Diet	CP-/Thr content	Mean liver weight (g)	TDG activity (nmol/30 min/mg protein)			Thr efficiency (bc^{-1})**
			Aminoacetone	Glycine	Total activity	
A	18.5% CP	34.2	2.58 [a]	1.71 [a]	4.29 [a]	144[a]
	0.45% dThr*	±4.0	±1.16	±0.85	±1.43	±5
B	18.5% CP	37.8	2.78 [a]	1.49 [a]	4.27 [a]	126[b]
	0.60% dThr	±4.8	±1.25	±0.95	±1.75	±2
C	22.5% CP	40.8	3.90 [ab]	2.62 [a]	6.52 [a]	177[c]
	0.45% dThr	±2.0	±2.06	±1.61	±3.31	±6
D	22.5% CP	37.5	5.88 [b]	5.57 [b]	11.45 [b]	162[d]
	0.60% dThr	±4.9	±2.07	±1.36	±3.22	±5

Values are mean±SD (n = 6). * True digestible threonine (AminoDat 1.1, Degussa 1997).
** Calculated from N-balance data, exponential N-utilization model. Different superscripts within a row indicate significant differences ($p<0.05$; Tukey's test).

excluded. The effects on the efficiency of threonine utilization (bc^{-1}), calculated from results of chicken N-balance data within our N-utilization model for growing animals (Table 7) are not completely consistent with the results from *in vitro* catabolism in the chicken liver. The data of the efficiency of threonine utilization indicate the end of the limiting range of threonine after L-threonine supplementation at both protein levels. This conclusion was confirmed by a significant lower efficiency of threonine utilization (bc^{-1}) and supports the sensitive reaction of N-utilization depending on the supply of limiting amino acid (Liebert and Gebhardt, 1980; Liebert et al., 1987; Moughan and Fuller, 2003).

Davis and Austic (1997) also suggested that the TDG activity could be additionally regulated by cellular concentrations of other amino acids. This suggestion is supported by observations that increasing levels of glutamic acid in the diet increased the hepatic TDG activity in pigs fed with a low threonine diet (Le Floc'h et al., 1994). Furthermore, TDG activity was increased by the addition of different amino acids or a mixture of indispensable amino acids lacking threonine (Davis and Austic, 1982, 1994).

In conclusion, the TDG activity in the liver of rat and growing chicken is not only modulated by the threonine concentration in the diet, but also by the protein concentration. Consequently, the interpretation of changes of the TDG activity in relation to the degradation rate of the limiting amino acid is difficult because of the interrelationship to the non-specific catabolism rate of other amino acids. The efficiency of threonine utilization derived from N-balance data and calculated within the applied exponential N-utilization model is generally an indicator of the limiting range of a dietary essential amino acid (Liebert and Gebhardt, 1980; Liebert et al., 1987; Liebert, 1995; Thong and Liebert, 2004; Liebert, 2008; Wecke and Liebert, 2010). The response of this parameter in relation to the dietary threonine supply was more pronounced under different dietary conditions, mainly in growing chicken. *In vitro* determination of the liver TDG activity is useful as an additional metabolic information but has to be evaluated under the specific experimental conditions in relation to other dietary factors which are involved in modulating the activity of this enzyme.

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