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Effects of dietary protein quality on energy metabolism and thyroid hormone status in growing pigs

Dedicated to Prof. Dr. habil. agr. H. Hagemeyer on the occasion of his 65th birthday

Summary

To estimate long-term effects of dietary protein quality on energy metabolism and thyroid hormone status in growing pigs two experiments were carried out, each using 6 growing German Landrace barrows (40 to 90 kg body weight (BW)) per treatment group, which were fed semisynthetic isoenergetic diets based on either casein or soy protein isolate at 1875 kJ ME/(kg BW^{0.62} x d). Casein was tested with (CAS+) amino acid (AA) supplementation (methionine + cystine, threonine, tryptophane) and soy protein isolate was tested without (SPI-) AA supplementation at the recommended protein supply of 100% (normal protein level (NP)) and at a protein supply of 50% of NP. During experiments pigs were housed individually in metabolic cages at 23 ± 1°C. At both protein supply levels, SPI- in comparison to CAS+ caused a lower protein energy retention (PER), which was compensated mainly by an increased fat energy retention (FER). The reduction of the protein supply to 50% caused a lower PER by 30 to 50% in both dietary qualities, which was compensated by a significantly higher FER. However, the heat production (HP) was neither affected by the protein quality nor by the quantity, and resulted in nearly similar values of 60% of ME intake. The thyroid hormone concentrations were dependent primarily on the amount of protein supply, and after decrease of supply to 50% secondly on the dietary protein quality. The increased thyroid hormone concentrations at the 50% protein level were in euthyroid range of pigs and obviously not associated with HP.

Key Words: protein quality, soy protein isolate, casein, thyroid hormones, energetic efficiency, pigs

Zusammenfassung

Titel der Arbeit: Effekte der Nahrungsproteinqualität auf den Energieumsatz und den Schilddrüsenhormonstatus wachsender Schweine

Um Langzeiteffekte von Nahrungsproteinqualitäten auf den Energieumsatz und den Schilddrüsenhormonstatus wachsender Schweine zu bestimmen, wurden 2 Versuche mit jeweils 6 wachsenden Börgen der Deutschen Landrasse (40 bis 90 kg LM) je Versuchsgruppe durchgeführt. Die Tiere wurden mit semisynthetischen, isoenergetischen Diäten (1875 kJ ME/(kg BW^{0.62} x d)) gefüttert, die Casein oder Sojaproteinisolat als alleinige Proteinquelle enthielten. Casein (CAS+) wurde mit Aminosäureergänzung (Methionin + Cystein, Threonin, Tryptophan) und Sojaproteinisolat (SPI-) ohne AA-Ergänzung auf einer 100 %igen Proteinversorgungsstufe (normal protein level, NP) und einer 50 %igen (low protein level (LP)) getestet. Während der Versuche wurden die Tiere einzeln in Stoffwechselläufigen bei 23 ± 1°C gehalten. Auf beiden Proteinversorgungsstufen führte die Fütterung von SPI- im Vergleich zu CAS+ zu einer signifikant niedrigeren Proteinenergierehaltung (PER), die überwiegend durch eine höhere Fettenergierehaltung (FER) kompensiert wurde. Die Senkung der Proteinversorgung auf 50 % hatte für beide Proteinqualitäten eine 30 bis 50 % niedrigere PER zur Folge, welches durch eine höhere FER kompensiert wurde. Die Wärmeproduktion (WP) wurde weder durch die Proteinqualität noch durch die -quantität beeinflusst und resultierte in nahezu gleichen Werten von 60 % der Einnahme an ME. Die Schilddrüsenhormonkonzentrationen waren in erster Linie von der Proteinversorgungsstufe abhängig und erst nach Senkung der Proteingabe auf 50 % von der Qualität. Die erhöhten Schilddrüsenhormonwerte auf LP lagen im euthyroiden Bereich und waren mit der WP offensichtlich nicht verbunden.

Schlüsselwörter: Proteinqualität, Sojaproteinisolat, Casein, Schilddrüsenhormone, Gesamtenergieverwertung, Schweine

Introduction

Long-term feeding of diets, which meet the energy requirements for maintenance and growth, but not the requirement for essential amino acids (AA), decreases growth rate and protein deposition in growing animals. The excess of dietary energy, which cannot be deposited as protein can either be used for fat deposition (KEAGY et al., 1987) or dissipated as heat (TULP et al., 1979; GURR et al., 1980). Although several studies were carried out to measure energy balance under these conditions, only a few studies have attempted to investigate the regulatory factors of thermogenesis. It is known that thyroid hormones may play a role in mediating the thermogenic response to low protein diets.

Less research has been done in examining the effect of feeding dietary proteins with a low biological value on energy metabolism of growing animals. From previous data one cannot conclude unambiguously whether specific AA patterns are responsible for differences in efficiency of the utilization of metabolizable energy (ME). It is observed that long-term feeding of plant protein (e.g. soy protein, wheat gluten) with somewhat lower biological value, in comparison to high quality animal proteins (casein, egg protein) induced higher serum thyroid hormone levels, particularly higher total thyroxine (T_4) levels, in monogastric animals (CREE and SCHALCH, 1985; FORSYTHE, 1986; BARTH et al., 1988; SCHOLZ-AHRENS et al., 1990; POTTER et al., 1996). The question arises whether a higher T_4 level in AA deficient fed animals represents a true change in biological activity and does play a role in dissipating excess dietary energy.

The objective of the present study was to investigate the influence of dietary protein quality (AA pattern) on energy metabolism and thyroid hormone status, as well as to explore whether differences in energetic efficiency are associated with changes in circulating thyroid hormone levels.

Materials and methods

Animals and diets

Two separate experiments were carried out each with six castrated male pigs of the German Landrace, weighing between 30 and 90 kg. At the initial body weight (BW) of 30 kg animals were equipped with permanent vein catheters for stress-free blood sampling. During the experiments the pigs were housed individually in metabolic cages. To meet the thermic demands of the animals the environmental temperature at the start of the trials was kept at 25°C. With increasing body weight the temperature was decreased up to 22°C. The relative humidity was 60-70%. They were fed twice daily with semisynthetic, isoenergetic diets, which provided 2.5 times the maintenance requirement for metabolizable energy ($1875 \text{ kJ ME}/(\text{kg BW}^{0.62} \times \text{d})$). This corresponded to a daily feed intake of approximately 110g DM/kg BW^{0.62}. Water was offered to *ad libitum* intake. To enable a comparison with human nutrition, nutrient composition of the diets was similar to the composition of human diets in western industrial countries (protein, 8-18%; starch, 32-42%; sucrose, 20%; fat, 15%; cellulose, 7%; mineral and vitamin mix, 8%, wt/wt). Protein and starch content were

altered, depending on age and level of protein supply. Casein was supplemented with limiting essential AA (Met + Cys, Thr, Trp) to the level recommended by the GfEH (1987). Correspondingly, CAS+ was supplemented with 1.15 g Met, 0.58 g Thr and 0.46 g Trp per 16 g N. Soy protein isolate was tested without (SPI-) supplementation. In the SPI- treatment the AA Lys, Met + Cys, Thr and Trp, provided 74, 58, 75 and 73% of the recommended level, respectively. For pre-calculation of ME values in diets (kJ/kg DM), the equation of HOFFMANN et al. (1993) was used. Effects of the dietary protein quality were compared at normal protein level (NP, experiment 1) and at low protein level (LP, 50% of NP, experiment 2).

Treatments and experimental procedures

In both experiments, trial periods were performed to the same pattern: 10 days of pre-period and 8 days of main period with determinations of energy and protein balance (CN balance), including 4 days of measurements of gaseous exchange in the respiration chamber. During the main period pigs were kept in metabolic cages allowing daily collection of urine and faeces separately. The body weight of the animals was recorded weekly and health status was monitored by daily measuring of rectal temperature. Each experiment was performed as a cross-over trial, i. e. after the 2nd period the dietary proteins CAS+ and SPI- were replaced by one another. Pigs were given 21 days to adapt to the new experimental conditions and diets. Measurements of CN balance were done in all periods and determination of thyroid hormones in the 2nd and 4th period. For thyroid hormone determination blood samples (10 mL/day) were taken during the main period prior to the morning feeding. Each sample was allowed to clot for 12 hours, followed by centrifugation with 3000 x g at 4°C. Aliquots of sera were taken and stored at - 20°C until assayed for thyroid hormones.

Analytical methods

The energy content of feed, freeze-dried faeces and urine was determined with an adiabatic bomb calorimeter (C400; JANKE & KUNKEL GmbH, Staufen, Germany). The amino acids were analyzed by ion-exchange chromatography using an amino acid analyser BIOCHROM 20 (PHARMACIA-BIOTECH EUROPE GmbH, Freiburg, Germany). For all other analyses of feed, faeces and urine conventional methods of the Verband Deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA, 1988) were used.

The energy balance was measured by indirect calorimetry based on the carbon and nitrogen balances. Energy retention was calculated by using the factors given by BROWER (1965) and HOFFMANN and SCHIEMANN (1980). Heat production was calculated as difference between ME intake and energy retention.

Thyroid hormones, total thyroxine (T₄), total triiodothyronine (T₃) and the free forms, fT₄ and fT₃, were determined by radioimmunoassays (RIA-COAT, BYK-SANTEC DIAGNOSTICA GmbH & Co.KG, Dietzenbach, Germany).

Statistical analysis

Effects of protein sources within periods were evaluated by one-way ANOVA using

SPSS (Statistical Package for the Social Science, Version 7.5, Chicago, 1997). All the results presented in tables are mean values with standard deviations. Differences were considered to be significant at $p < 0.05$.

Results

Nutrients and energy of both diets were highly digestible (approximately 90%, data not shown). The apparent N digestibility was influenced by both the protein quality and quantity and ranged between 89.9 and 93.5 at NP and between 82.9 and 87.1% at the LP level, always with the lower values in the SPI- groups.

With the exception of the 2nd period of NP the differences in the intake of metabolizable energy between CAS+ and SPI- within periods were not significantly different (Table 1). Generally the intake of ME in SPI- fed pigs was slightly lower. Within the periods of NP the intake of protein per kg BW^{0.62} was not significantly different. At LP the intake of protein per kg BW^{0.62} within the periods was slightly but significantly lower for SPI- than for CAS+.

Both, protein quality and quantity, affected the daily weight gain of pigs (Table 1). At NP the values for CAS+ fed pigs in the 1st and 2nd period were 504 and 567 g, respectively, which was in both periods approximately 90 g higher than the daily gain of the SPI- pigs. After cross-over feeding, the difference between CAS+ and SPI- increased to 130 g/d in the 3rd period; in the 4th period the difference was 86 g/d. In all periods of NP the differences were statistically significant. Reduction of the protein content to 50% (LP) generally resulted in a reduced growth performance in both dietary groups. The daily gain of CAS+ fed pigs amounted from 302 g in the 1st to 494 g in the 4th period, and of SPI- fed pigs from 262 to 406 g, respectively. With the exception of the 3rd period, after cross-over feeding, differences in daily gain between CAS+ and SPI- groups remained significant. The final BW within the same time period was approximately 90 kg at the NP and approximately 60 kg at the LP feeding level.

At almost the same ME intake levels, protein energy retention (PER) was markedly affected by protein quality and quantity. In the 1st period of NP, feeding of SPI- resulted in a significantly lower PER than feeding of CAS+ (205 vs. 279 kJ). The values of PER in the 2nd period decreased for CAS+ by 27% and for SPI- by 18% compared to the 1st period. However, the difference between both protein sources was significant. After cross-over feeding, the values of PER for SPI- remained constant but the value for CAS+ increased to 221 kJ. In the 4th period the values of PER decreased further for both protein sources, however the differences between CAS+ and SPI- remained significant.

As the PER during growth decreased, the fat energy retention (FER) increased for CAS+ from 394 kJ in the 1st period to 715 kJ in the 4th period, and for SPI- from 447 to 642 kJ, respectively. Also, the total energy retention (ER) increased from 673 kJ for CAS+ and 651 kJ for SPI- in the 1st period to 882 and 785 kJ in the 4th period, respectively. Correspondingly, heat production (HP) decreased for both treatments. Although in all periods of NP feeding of SPI- as compared to CAS+ resulted in a significantly lower PER, no significant differences in values of FER (exception of 4th

Table 1

Energy and N balance data of growing pigs fed different dietary protein qualities and quantities (Means and standard deviations). (Energie- und N-Bilanzdaten wachsender Schweine bei Fütterung unterschiedlicher Nahrungsproteinqualitäten und -quantitäten (Mittelwerte und Standardabweichungen))

Period	Protein source	n animals	Mean BW ¹ (kg)	Weight gain (g/d)	ME intake kJ/(kg BW ^{0.62} x d)	Protein intake g/(kg BW ^{0.62} x d)	Energy retention (ER)	Protein ER kJ/(kg BW ^{0.62} x d)	Fat ER	Heat production
Experiment 1: Normal protein level, NP (Protein supply: 100%)										
1	CAS+	6	41.5 ± 4.1	504 ± 20 ^a	1891 ± 49 ^a	17.2 ± 1.0 ^a	673 ± 94 ^a	279 ± 25 ^a	394 ± 86 ^a	1217 ± 67 ^a
	SPI-	6	40.3 ± 2.8	415 ± 15 ^b	1837 ± 52 ^a	17.4 ± 0.6 ^a	651 ± 56 ^a	205 ± 9 ^b	447 ± 50 ^a	1186 ± 58 ^a
2	CAS+	6	51.9 ± 3.6	567 ± 38 ^a	1905 ± 28 ^a	15.5 ± 0.8 ^a	748 ± 45 ^a	205 ± 6 ^a	542 ± 43 ^a	1157 ± 65 ^a
	SPI-	6	49.6 ± 2.6	469 ± 21 ^b	1834 ± 32 ^b	15.6 ± 0.4 ^a	709 ± 76 ^a	168 ± 8 ^b	541 ± 74 ^a	1125 ± 59 ^a
3	CAS+	6	74.2 ± 2.6	726 ± 31 ^a	1930 ± 33 ^a	14.5 ± 0.3 ^a	821 ± 45 ^a	221 ± 16 ^a	600 ± 52 ^a	1109 ± 61 ^a
	SPI-	6	73.0 ± 4.8	595 ± 23 ^b	1897 ± 36 ^a	14.6 ± 0.7 ^a	779 ± 50 ^a	167 ± 7 ^b	612 ± 44 ^a	1119 ± 72 ^a
4	CAS+	5*	88.3 ± 3.2	763 ± 21 ^a	1919 ± 26 ^a	13.5 ± 0.2 ^a	882 ± 46 ^a	168 ± 5 ^a	715 ± 48 ^a	1037 ± 39 ^a
	SPI-	5*	83.3 ± 4.1	677 ± 30 ^b	1889 ± 46 ^a	13.7 ± 0.5 ^a	785 ± 37 ^b	143 ± 14 ^b	642 ± 38 ^b	1104 ± 51 ^a
Experiment 2: Low protein level, LP (Protein supply: 50% of NP)										
1	CAS+	6	32.2 ± 1.5	302 ± 28 ^a	1924 ± 60 ^a	9.1 ± 0.1 ^a	751 ± 73 ^a	144 ± 7 ^a	607 ± 69 ^a	1173 ± 48 ^a
	SPI-	6	32.1 ± 0.8	262 ± 31 ^b	1917 ± 49 ^a	9.6 ± 0.1 ^b	740 ± 73 ^a	89 ± 12 ^b	651 ± 68 ^a	1178 ± 68 ^a
2	CAS+	6	38.7 ± 3.1	343 ± 28 ^a	1921 ± 51 ^a	8.9 ± 0.1 ^a	783 ± 88 ^a	148 ± 9 ^a	635 ± 81 ^a	1137 ± 42 ^a
	SPI-	6	37.7 ± 0.8	298 ± 48 ^b	1875 ± 43 ^a	9.6 ± 0.2 ^b	724 ± 39 ^a	96 ± 10 ^b	629 ± 35 ^a	1150 ± 57 ^a
3	CAS+	6	49.3 ± 1.3	358 ± 33 ^a	1831 ± 35 ^a	8.0 ± 0.1 ^a	699 ± 44 ^a	114 ± 5 ^a	585 ± 40 ^a	1132 ± 57 ^a
	SPI-	4**	51.1 ± 1.6	337 ± 17 ^a	1812 ± 9 ^a	8.4 ± 0.2 ^b	687 ± 15 ^a	83 ± 5 ^b	604 ± 12 ^a	1125 ± 17 ^a
4	CAS+	6	57.2 ± 2.4	494 ± 54 ^a	1816 ± 61 ^a	7.9 ± 0.1 ^a	742 ± 53 ^a	119 ± 2 ^a	623 ± 52 ^a	1074 ± 57 ^a
	SPI-	4**	58.3 ± 1.7	406 ± 41 ^b	1828 ± 40 ^a	8.4 ± 0.2 ^b	720 ± 55 ^a	82 ± 7 ^b	638 ± 48 ^a	1108 ± 33 ^a

Protein sources were casein with amino acid supplementation (CAS+) and soy protein isolate without amino acid supplementation (SPI-). Experiments were cross over trials, i. e. after two periods dietary proteins CAS+ and SPI- were replaced by each other. ¹Mean body weight (kg) of the group at middle of the respective period.

^{a,b} Means with different superscripts within a period and within one column are significantly different (p < 0.05).

*One animal was taken out of experiments due to health problems; **Two animals were taken out of experiments due to health problems.

period), as well as of HP, between CAS+ and SPI- were determined.

In Table 2 the partition of ME into PER and FER as well as into ER (total efficiency of utilization of ME) and HP in % are calculated. With exception of the 4th period, feeding of SPI- in comparison to CAS+ resulted in a significantly lower proportion of ME in PER; values of FER/ME and ER/ME as correspondingly HP/ME were not significantly different but always on an average slightly higher for SPI-. There was a decrease of PER/ME and an increase of FER/ME for both dietary groups during growth of the animals. In the periods 1 to 3, CAS+ fed pigs showed significantly higher values of PER/ER than SPI-fed pigs. During growth, as the values of PER/ER decreased the values of FER/ER increased. Additionally, the differences between CAS+ and SPI- diminished with increasing BW, however in the 4th period no significant differences were observed.

The reduction of protein supply to 50% (LP) resulted in a similar HP than at NP (Table 1). At LP markedly lower PER (50 to 60%) and higher FER (30 to 40%) than at NP in both dietary groups were observed. During growth PER decreased slightly for CAS+ as well as for SPI-, however the differences between both protein sources remained significant. In all periods of LP for FER and HP no significant differences between CAS+ and SPI- were observed.

The proportions of HP in ME (Table 2) between CAS+ and SPI- were not significantly different in all periods of LP; both dietary groups showed an average value of 60%. Feeding of SPI- resulted in a significantly lower proportion of PER in ME than feeding CAS+, FER/ME was not affected by the dietary treatment. There was a decrease of PER/ME and an increase of FER/ME for both dietary groups during growth of pigs. In all periods of LP CAS+ resulted in a significantly higher ratio of PER/ER and in a lower ratio of FER/ER than SPI-. During growth the values of PER/ER decreased for CAS+ from 19.2 to 16.0%; for SPI- the values remained constant at about 12%.

Thyroid hormones

Concentrations of thyroid hormones in the serum measured at both protein levels in the 2nd and 4th fattening period are summarized in Table 3. Thyroid hormone levels were constant within periods, which allowed to calculate an average for each variable (T_4 , T_3 , fT_4 , fT_3) for each period.

At NP, serum concentrations of thyroid hormones were not affected by the dietary protein quality. The ratio of T_3/T_4 was not affected by the dietary protein quality or by the period. In the 4th period similar thyroid hormone concentrations were determined as in the 2nd period.

At LP, serum concentrations of thyroid hormones were affected by the dietary protein quality. In the 2nd period for SPI- in comparison to CAS+ not significantly but slightly higher concentrations of T_4 (56.28 vs. 50.84 nmol/L) and significantly higher concentrations of fT_4 (16.15 vs. 11.91 pmol/L) were observed. In contrast, significantly lower values of T_3 (1.57 vs. 2.32 nmol/L) and fT_3 (0.31 vs. 0.75 pmol/L) were determined compared to the CAS+. Similar observations were made in the 4th period.

The reduction of protein supply to 50% (LP) caused a markedly higher T_4 concent-

Table 2

Partition of metabolizable energy (ME) into protein energy retention (PER), fat energy retention (FER), total energy retention (ER) and heat production (HP) as well as ratios of PER to ER and FER to ER in growing pigs fed different protein qualities and quantities (Means and standard deviations). (Aufteilung der umsetzbaren Energie (ME) in Proteinenergierehaltung (PER), Fettenergierehaltung (FER), Gesamtenergierehaltung (ER) und Wärmeproduktion (HP) sowie die Verhältnisse von PER zu ER und FER zu ER in wachsenden Schweinen bei der Fütterung unterschiedlicher Nahrungsproteinqualitäten und -quantitäten (Mittelwerte und Standardabweichungen))

Period	Protein source	n animals	PER/ME (%)	FER/ME (%)	ER/ME (%)	HP/ME (%)	PER/ER (%)	FER/ER (%)
Experiment 1: Normal protein level, NP (Protein supply 100%)								
1	CAS+	6	14.7 ± 1.1 ^a	20.8 ± 4.2 ^a	35.6 ± 4.3 ^a	64.4 ± 4.3 ^a	41.8 ± 5.1 ^a	58.2 ± 5.1 ^a
	SPI-	6	11.1 ± 0.3 ^b	24.3 ± 2.6 ^a	35.4 ± 2.9 ^a	64.6 ± 2.9 ^a	31.5 ± 1.9 ^b	68.5 ± 2.0 ^b
2	CAS+	6	10.8 ± 0.4 ^a	28.5 ± 2.6 ^a	39.3 ± 2.7 ^a	60.7 ± 2.7 ^a	27.6 ± 1.6 ^a	72.4 ± 1.6 ^a
	SPI-	6	9.1 ± 0.5 ^b	29.5 ± 3.6 ^a	38.6 ± 3.7 ^a	61.4 ± 3.7 ^a	23.8 ± 2.1 ^b	76.2 ± 2.1 ^b
3	CAS+	6	11.5 ± 0.8 ^a	31.1 ± 3.0 ^a	42.5 ± 2.6 ^a	57.5 ± 2.6 ^a	27.0 ± 2.9 ^a	73.0 ± 3.0 ^a
	SPI-	6	8.8 ± 0.5 ^b	32.3 ± 2.6 ^a	41.1 ± 3.0 ^a	58.9 ± 3.0 ^a	21.5 ± 0.8 ^b	78.5 ± 0.8 ^b
4	CAS+	5*	8.2 ± 1.3 ^a	37.4 ± 2.1 ^a	45.6 ± 2.1 ^a	54.4 ± 2.1 ^a	18.0 ± 2.8 ^a	82.0 ± 2.8 ^a
	SPI-	5*	7.1 ± 1.1 ^a	34.8 ± 2.8 ^a	41.1 ± 2.1 ^b	58.1 ± 2.1 ^b	17.1 ± 3.1 ^a	82.9 ± 3.1 ^a
Experiment 2: Low protein level, LP (Protein supply: 50% of NP)								
1	CAS+	6	7.5 ± 0.2 ^a	31.5 ± 3.0 ^a	39.0 ± 3.0 ^a	61.0 ± 3.0 ^a	19.2 ± 0.9 ^a	80.8 ± 1.6 ^a
	SPI-	6	4.6 ± 0.6 ^b	34.0 ± 3.4 ^a	38.6 ± 3.5 ^a	61.4 ± 3.5 ^a	12.0 ± 1.6 ^b	88.0 ± 1.6 ^b
2	CAS+	6	7.7 ± 0.3 ^a	33.0 ± 3.4 ^a	40.7 ± 3.5 ^a	59.3 ± 3.5 ^a	19.2 ± 1.8 ^a	80.9 ± 1.3 ^a
	SPI-	6	5.1 ± 0.5 ^b	33.6 ± 0.7 ^a	38.7 ± 2.3 ^a	61.3 ± 2.3 ^a	13.2 ± 1.2 ^b	86.7 ± 1.2 ^b
3	CAS+	6	6.2 ± 0.2 ^a	32.0 ± 2.3 ^a	38.2 ± 2.5 ^a	61.8 ± 2.5 ^a	16.2 ± 0.4 ^a	83.7 ± 0.7 ^a
	SPI-	4**	4.6 ± 0.3 ^b	33.4 ± 0.7 ^a	37.9 ± 0.9 ^a	62.1 ± 0.9 ^a	12.0 ± 0.5 ^b	88.0 ± 0.5 ^b
4	CAS+	6	6.5 ± 0.2 ^a	34.3 ± 2.6 ^a	40.7 ± 2.6 ^a	59.2 ± 2.6 ^a	16.0 ± 0.9 ^a	84.0 ± 1.2 ^a
	SPI-	4**	4.5 ± 0.3 ^b	34.9 ± 2.0 ^a	39.3 ± 2.4 ^a	60.6 ± 2.4 ^a	11.4 ± 0.3 ^b	88.6 ± 0.3 ^b

Protein sources were casein with amino acid supplementation (CAS+) and soy protein isolate without amino acid supplementation (SPI-). Experiments were cross over trials, i. e. after two periods dietary proteins CAS+ and SPI- were replaced by each other.

^{a,b} Means with different superscripts within a period and within one column are significantly different ($p < 0.05$).

* One animal was taken out of experiments due to health problems; ** Two animals were taken out of experiments due to health problems.

Table 3

Concentration of thyroid hormones (T_4 , T_3 , fT_4 and fT_3)¹ in the serum of growing pigs fed different dietary protein qualities and quantities (Means and standard deviations). (Schilddrüsenhormonkonzentration (T_4 , T_3 , fT_4 and fT_3)¹ im Serum wachsender Schweine bei Fütterung unterschiedlicher Nahrungsproteinqualitäten und -quantitäten (Mittelwerte und Standardabweichungen))

Period	Protein source	n pigs	Total T_4 (T_4) nmol/L	Free T_4 (fT_4) pmol/L	Total T_3 (T_3) nmol/L	Free T_3 (fT_3) pmol/L	T_3/T_4 %
Experiment 1: Normal protein level, NP (Protein supply: 100%)							
2	CAS+	6	37.71 ± 5.76 ^a	13.52 ± 2.01 ^a	1.69 ± 0.32 ^a	0.43 ± 0.25 ^a	4.5 ± 1.0 ^a
	SPI-	6	35.58 ± 6.06 ^a	12.51 ± 2.57 ^a	1.63 ± 0.41 ^a	0.72 ± 0.33 ^a	4.6 ± 1.3 ^a
4	CAS+	5*	38.02 ± 6.93 ^a	12.17 ± 2.40 ^a	1.63 ± 0.30 ^a	0.45 ± 0.30 ^a	4.3 ± 0.8 ^a
	SPI-	5*	38.80 ± 6.41 ^a	14.67 ± 2.00 ^a	1.58 ± 0.27 ^a	0.59 ± 0.34 ^a	4.1 ± 0.8 ^a
Experiment 2: Low protein level, LP (Protein supply: 50% of NP)							
2	CAS+	6	50.84 ± 7.93 ^a	11.91 ± 2.23 ^a	2.32 ± 0.40 ^a	0.75 ± 0.33 ^a	4.6 ± 1.2 ^a
	SPI-	4**	56.28 ± 12.20 ^a	16.15 ± 4.10 ^b	1.57 ± 0.31 ^b	0.31 ± 0.18 ^b	2.8 ± 0.7 ^b
4	CAS+	6	43.62 ± 8.62 ^a	9.88 ± 1.89 ^a	2.19 ± 0.43 ^a	1.04 ± 0.45 ^a	5.0 ± 1.3 ^a
	SPI-	4**	50.53 ± 7.00 ^a	16.17 ± 4.10 ^b	1.21 ± 0.21 ^b	0.40 ± 0.25 ^b	2.4 ± 0.6 ^b

Protein sources were casein with amino acid supplementation (CAS+) and soy protein isolate without amino acid supplementation (SPI-). Experiments were cross over trials, i. e. after two periods dietary proteins CAS+ and SPI- were replaced by each other. ^{a,b} Means with different superscripts within a period and within one column are significantly different ($p < 0.05$); ¹ T_4 , total thyroxine; T_3 , total triiodothyronine; fT_3 , free triiodothyronine. fT_4 , free thyroxine

*One animal was taken out of experiments due to health problems;

**Two animals were taken out of experiments due to health problems.

ration (30%) for both protein qualities, and a higher T_3 concentration (30%) for CAS+. Consequently, the T_3/T_4 ratio for CAS+ resulted in similar values of approximately 4.4% as at NP; the ratio for SPI- was decreased to 2.8% in the 2nd and to 2.4% in the 4th period.

Discussion

It is known that thyroid hormones may play a role in mediating the thermogenic response to low protein diets. The decreased energetic efficiency observed in growing rats and pigs fed low protein diets is often associated with a marked rise in T_3 concentration (TULP et al., 1979, ROTHWELL et al., 1983, GURR et al., 1980), which is frequently interpreted as an adaptive diet-induced thermogenesis. Some energy dissipating systems such as the hepatic mitochondrial α -GP shuttle (TYZBIR et al., 1981, SAWAYA and LUNN, 1985, KEAGY et al., 1987), as well as the thermogenic activity of brown adipose tissue (ROTWELL et al., 1983) are sensitive to the thyroid hormone status. Because dietary proteins with a lower biological value induce an increase in T_4 levels, we propose that similar to protein deficient diets the excess of dietary energy, which can not be deposited as protein, could also be dissipated as heat through increased thermogenesis.

In the present study at LP the T_4 concentrations were slightly but the fT_4 concentrations were significantly higher in SPI- than in CAS+ fed pigs within both periods. These findings are in accordance with literature data (CREE and SCHALCH, 1985; FORSYTHE, 1986; BARTH et al., 1988; SCHOLZ-AHRENS et al., 1990; POTTER et al., 1996). At NP, serum concentrations of all thyroid hormones were not affected by the dietary protein quality. The latter finding is surprising, because BARTH et al. (1988) and SCHOLZ-AHRENS et al. (1990) have shown that feeding pigs with diets similar in composition to the NP diets, providing 18, 52 and 30% of ME from protein, carbohydrate and fat respectively, resulted in significantly higher T_4 concentrations in SPI- fed pigs. Reasons for the contrary endocrine response may be due to different pig breeds, age and sex of animals. In the present study growing castrated male pigs of the German Landrace were fed isoenergetic diets, which provided for 2.5 times the maintenance requirement of ME. BARTH et al. (1988) and SCHOLZ-AHRENS et al. (1990) used adult female Göttingen miniature pigs fed isoenergetic diets at the maintenance requirement of ME. However, BARTH et al. (1988) obtained in experiments with growing minipigs fed 15% soy protein isolate or casein higher values of T_4 and fT_4 in the SPI- group, but the difference was statistically not significant. These findings correspond better to our findings at the NP level.

The present results indicated that both CAS+ and SPI- fed pigs responded similarly to the low protein feeding regime by exhibiting an increase in total T_4 concentration of 30%. Interestingly, the T_3 and fT_3 levels were only increased in the CAS+ fed pigs. However, we expected results in the opposite direction. But recently, similar inverse relationships between T_3 concentration and casein diets have been reported by POTTER et al. (1996), who carried out experiments with hamsters. Our findings are partially in disagreement with other literature data (TULP et al. 1979, ROTHWELL et al., 1983), which showed that consumption of low protein diets is associated with an increase in T_3 concentration and mostly with no effect on the T_4 level. However, interpretation is made difficult, if animals were fed diets to *ad libitum* intake. In contrast to our findings such data do not allow to conclude unambiguously whether protein supply is responsible for differences in thyroid hormone levels. It is possible that the increased T_3 level in LP diets was a response to an elevation in food and energy intake (DANFORTH et al., 1979)

Our observations of increased serum T_4 concentrations are consistent with findings of ORIEN et al. (1979), who estimated elevations in T_4 concentrations in young rats fed protein restricted diets (8%) of a normal energy density in comparison to high protein diets (22%). Because T_4 is synthesised exclusively from the thyroid gland it may be suspected that the observed higher T_4 levels may indicate an increase in thyroid secretion induced by dietary protein deficiency. In contrast, ATINMO et al. (1978) observed lower serum T_4 concentrations in pigs after feeding low protein diets. Furthermore, the question arises why it comes to contrary endocrine response in T_4 concentration after feeding low protein diets. BERGNER (1989) found lower thyroid secretion rates (TSR) in pigs and rats after feeding proteins with a lower biological value. He concluded that the T_4 concentration in the blood depends on the thyroid secretion rates and the T_4 utilization of tissue. From this point of view it can not be

concluded that higher serum T_4 concentrations exclusively derive from a higher TSR. The ratio T_3/T_4 , as a measure of the peripheral deiodination of T_4 , should also be interpreted critically. NOWAK and SLEBODZINSKI (1986) proved that the greatest proportion of the daily T_3 production derived from peripheral monodeiodination of T_4 with values ranging between 70 and 80%. In the present study the T_4 levels at LP were increased in both dietary groups, the CAS+ groups resulted in similar values of T_3/T_4 ratio at both protein levels. Apparently at LP, more T_3 is derived from T_4 in the periphery in CAS+ fed pigs, which is also covered by higher T_3 values. The T_3 levels for SPI- were similar at both protein levels with the consequence of a 50% reduction in the T_3/T_4 ratio at LP. In general, a higher T_4 level is followed by a higher T_3 level (VOSBERG and WAGNER, 1991). A reason for the contrary endocrine response of SPI- at LP may be due to the lower protein quality of SPI-. As described by LAUTERIO and SCANES (1987), phenylalanine and tyrosine deficiency have been found to decrease circulating T_3 concentrations. Since SPI- contains approximately 35% less of both AA than CAS+, the lack of an increase in T_3 concentration following an increased T_4 level in SPI-, in comparison to CAS+, may be explained by the deficiency of these two AA in SPI- at LP.

In summary, our findings indicate that the circulating concentration of thyroid hormones depends primarily on the protein supply and after a decrease of supply to 50% secondly on the dietary protein quality.

In general, long term feeding of dietary proteins with a lower biological value leads to significant lower growth performance, i.e. protein deposition, than feeding dietary proteins with a higher value (SALTER et al., 1990; ROY et al., 1997; SÉVE et al., 1997). The present results indicate changes in the same manner when feeding SPI- with the lower biological value in comparison to CAS+ in all periods at both protein levels. The excess of dietary energy, which cannot be deposited as protein, can either be used for fat deposition (KEAGY et al., 1987) or dissipated as heat (TULP et al., 1979; GURR et al., 1980).

In the present study the total efficiency of utilization of ME (energy retention/ME intake) within periods at both protein levels was not significantly different between CAS+ and SPI-. Independent on the protein quality and quantity, pigs retained 38 to 42% of ME. The slightly lower efficiency of utilization of ME (36 vs. 40%) in the 1st period of NP, in both feeding groups, was caused by the higher PER in relation to FER (see table 2). However, in general, our findings are in accordance with the data of a review presented by BERGNER and HOFFMANN (1996), who indicated values of 40 to 45% for growing finishing pigs.

Under the conditions of feeding isoenergetic diets, which provided 2.5 times the maintenance requirement of metabolizable energy ($1875 \text{ kJ ME}/(\text{kg BW}^{0.62} \times \text{d})$), the pigs fed SPI- retained less protein energy per unit metabolic BW compared to pigs fed CAS+. However, with one exception, SPI- feeding neither resulted in a significantly higher FER nor in a significantly higher HP. There are several reasons for no observed differences in both of these parameters with regards to CAS+ and SPI-. First there is, on average, a 3% lower ME intake in SPI fed pigs. Secondly, the changes in PER between SPI- and CAS+ are significant, however, they are expressed as values with an

average of $46 \text{ kJ}/(\text{BW}^{0.62} \times \text{d})$. When taking the value of the standard deviation (on average $53 \text{ kJ}/(\text{BW}^{0.62} \times \text{d})$) of HP into consideration, it is evident that the standard deviation in HP is equal or even higher than the difference in PER between CAS+ and SPI-, therefore no significant differences in FER and HP could be expected. In addition the higher excess of ME in SPI-, in comparison to CAS+, seems to be divided into both FER and HP.

Analogous experiments with growing rats fed low protein diets based on SPI- or CAS+ (KLEIN et al., 2000) are not consistent with our findings of a similar energetic efficiency in SPI- and CAS+ fed pigs. The SPI- fed rats retained both less protein energy and fat energy in comparison to CAS-fed rats.

Several experimental results reported in the literature (e.g., MÜLLER and KIRCHGESSNER, 1979; SCHIEMANN et al., 1983; reviewed by KLEIN and HOFFMANN, 1989) demonstrated that the additional energy costs for protein deposition compared to fat deposition are relatively high. The protein deposition is connected to the processes of protein turnover, in which the additional energy costs are mainly caused by higher synthesis rates. MILLWARD et al. (1976) calculated a value of energy costs (ME) for synthesis, which is dissipated as heat, of $0.15 \text{ kJ}/\text{kJ}$ and $3.6 \text{ kJ}/\text{g}$ protein synthesized, respectively. This value is based on the generally accepted energy cost of 5 moles ATP per mole of amino acid incorporated in the peptide chain, and represents the minimum cost of protein synthesis. When considering the relatively high standard deviation in HP, the question arises whether there were differences in the protein synthesis between SPI- and CAS-fed pigs, which produced measurable differences in HP. In the present study measurements of energy balance and protein turnover were carried out simultaneously (SAGGAU et al., 2000). The comparison between CAS+ and SPI- demonstrated that in CAS+ fed pigs, the higher protein deposition was realized by both a higher synthesis and degradation, e.g., enhanced protein turnover. Under consideration of calculated values for minimum energy costs (ME) of protein synthesis (MILLWARD et al., 1976) and the observed differences in protein synthesis between the both dietary groups, no measurable effect on HP can be expected between CAS+ and SPI- within the periods of both dietary levels.

FULLER et al. (1987 a, b) also carried out trials with growing pigs fed daily a constant energy supply with a low in comparison to a high protein level and a variation of protein quality by altering the level of the first limiting AA, lysine, at both protein supply levels. Their data support our conclusion that there was no change in heat production after feeding an AA deficient diet.

As mentioned above, higher T_4 and fT_4 levels were estimated in SPI- than in CAS+ fed pigs at NP. In addition, both SPI- and CAS+ fed pigs responded similarly to LP by exhibiting an increase in total T_4 concentration of 30%. However we could not establish any relationship between increased thyroid hormone concentrations and heat production. HILLGARTNER and ROMSOS (1987) postulated that higher thyroid concentrations resulting from consumption of low protein diets are not directly responsible for activation of adaptive thermogenesis. HILLGARTNER and ROMSOS (1987) carried out experiments with rats fed diets containing 5, 8 or 22% CAS. The T_3 level was increased in the same manner when fed 5 or 8 in comparison to 22% CAS

diets but the efficiency of energy retention was reduced in rats fed 5% CAS, whereas no significant change was observed in rats fed 8%. From these findings, the authors concluded that thyroid hormones play only a permissive role in dissipation of energy in growing rats.

In a recent paper, MOROVAT and DAUNCEY (1998) analysed the thyroid hormone status in growing pigs of the Large White breed after modification of feed intake. Pigs with a total T_4 concentration of 37.9 ± 3.9 nmol/L were described as euthyroid and those with a concentration of 70 nmol/L and higher were considered hyperthyroid. From these findings, we concluded that total T_4 concentration (50 to 56 nmol/L) at LP were slightly increased but still euthyroid.

Furthermore, the question arises of how an excess of dietary energy can increase either fat deposition or heat production. GURR et al. (1980) demonstrated both metabolic pathways in growing pigs. There were, however, essential differences to our trials. GURR et al. fed either restricted amounts of a high protein diet (26%) or to *ad libitum* amounts of a low protein diet (2%) to pigs of 6 and 20 kg BW. In all cases, pigs fed low protein diets consumed approximately three times as much as pigs fed high protein diets. In contrast we fed similar restricted amounts of isoenergetic diets per unit metabolic body weight in all fattening periods of NP and LP which enabled a comparison due to protein intake quantity and/or quality differences alone. GURR et al. (1980) demonstrated that in 20 kg pigs, almost 70% of the energy excess caused by feeding the low protein diet was deposited in the carcass as fat, which is in accordance to our findings. In the 6 kg pigs fed low protein diets, changes in body energy content accounted for only a small fraction (27%) of the total energy intake, a large difference in energy expenditure was seen between these animals and the high protein group, which GURR et al. (1980) attributed to differences in dietary-induced thermogenesis. In addition, possible metabolic indicators of diet induced thermogenesis were investigated in the group of 6 kg pigs. At the low protein supply these pigs showed higher plasma T_3 levels and hepatic mitochondrial α -glycerophosphate dehydrogenase activity. It must be taken into account that at LP the ratio of protein energy to total energy intake was drastically reduced and corresponded to a supply as in Kwashiorkor patients (COWARD and LUNN, 1981; SCHOPPE, 1988). In these metabolic situation very young pigs seemed to dissipate the excess of energy mainly as heat through increased thermogenesis. It is apparent from the study of DANFORTH et al (1979) on humans that the intake of high caloric amounts, e.g. above the energetic requirement, also increases T_3 concentrations, which may be associated with an increased thermogenesis. ROTHWELL et al. (1983) speculated that the thermogenic activity of brown adipose tissue (BAT) may play an important role in diet-induced thermogenesis when low protein diets are fed to rats. The BAT contains a tissue-specific mitochondrial uncoupling protein (UCP), which is sensitive to thyroid hormones. KEAGY et al. (1987) postulated that protein-deficient chickens cannot dispose a surplus of dietary energy by this mechanism, because most birds apparently lack BAT. It is known, that pigs also do not contain BAT (TRAYHURN et al., 1989) and therefore they cannot use this mechanism. However, recently in white adipose tissue a similar tissue-specific mitochondrial uncoupling protein (UCP 2) was discovered (FLEURY et al., 1997).

It is concluded that, generally, HP was neither clearly affected by the protein quality nor by the quantity and resulted almost in identical values of 60% of ME intake. The increased thyroid hormone levels at LP level were still in euthyroid range of pigs and obviously not associated with HP.

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In einer Zeit zunehmender Technisierung und elektronischer Medien in allen Arbeits- und Lebensbereichen erhält die Beziehung vieler Menschen zu Haustieren einen immer größeren Stellenwert. Das gilt in besonderer Weise für das Pferd. Durch das vorliegende Buch ist der Verlag einmal mehr diesem Bedürfnis in gelungener Weise nachgekommen. Dieses Buch zeichnet ein Gesamtbild der faszinierenden Vielfalt vorhandener Pferderassen. In der gelungenen Kombination von Wort und Bild vermittelt es Wissenswertes über mehr als 300 Rassen. Es ist nicht einfach die Pferde- und Ponyrassen, so sie denn über ein Stutbuch verfügen und Pferdetyphen, die nach ihrem Nutzungszweck definiert werden, zu klassifizieren. Das war auch nicht das Ziel des Autors, dem es darum ging dem Leser in gebotener Kürze einen umfassenden Überblick über ca. 320 Pferde- und Ponyrassen der Welt zu geben und sie in diesem Bilderatlas in alphabetischer Reihenfolge aufzuführen. Dass es ihm dabei gelang dieses Weltrassenspektrum, neben den wichtigsten Angaben zu Exterieur, Verbreitung, Leistungsmerkmalen, Verwendung und Zuchtgeschichte, meist auch durch farbige Abbildungen vorzustellen, ist ein besonderes Verdienst. Es macht dieses Buch daher auch für Leser interessant, die Freude an schönen Tierbildern haben und nicht direkt mit der Pferdehaltung liiert sind.

In den ersten einführenden, allgemeinen Buchabschnitten werden Ursprung des Pferdes, das Pferd in vorgeschichtlicher Zeit, das frühgeschichtliche Pferd und die Entwicklung der Rassen vorgestellt. Informationen über „Das Pferd macht Weltgeschichte“ und „Das Pferd in der Kulturgeschichte“ beschließen diesen mit einer Fülle von Informationen zusammengestellten Buchteil. Sie belegen, dass im Vergleich zu den meisten anderen Haustieren, das relativ spät domestizierte Pferd die Menschen bis in die Gegenwart in unterschiedlicher Weise auf allen ihren Wegen begleitet hat und ihnen ein zuverlässiger Partner geworden ist. Im Hauptteil des Buches werden die Rassen vorgestellt. Der Leser findet für jede Rasse in komplexer, übersichtlicher und fundierter Weise, sich auf das Wesentliche beschränkende Informationen. Die Textaussagen werden durch ausdrucksvolle Farbfotos unterstützt. Ergänzt wird dieser farbige Bildatlas durch ein umfangreiches internationales Adressenverzeichnis sowie eine Literaturübersicht.

Dieses Buch hält nicht nur für Pferdezüchter, -freunde und -liebhaber eine Fülle von sorgfältig zusammengetragenen Informationen bereit. Es verschafft einen umfassenden Überblick über die bemerkenswerte Vielfalt des Weltrassenspektrums. Als unverzichtbares Nachschlagewerk für jeden ambitionierten Pferdefreund ist es uneingeschränkt Tierliebhabern und vielen weiteren interessierten Lesern zu empfehlen.

ERNST RITTER, Dummerstorf