

## Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization

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An experiment was conducted to investigate the effects of branched-chain amino acid (BCAA) interactions on their utilization by growing pigs and the effects of excessive amounts of BCAA (leucine, isoleucine, valine) on the utilization of methionine. A semipurified diet containing 100 g crude protein/kg with a balanced amino acid pattern was prepared using casein supplemented with free amino acids. Three further diets were made by reducing the concentration of methionine + cyst(e)ine, valine or isoleucine by 20%. Each of these four diets was then supplemented with leucine (50% excess) or a mixture of BCAA (50% excess of each but excluding the limiting amino acid). All diets were isoenergetic and were made isonitrogenous by replacement of glutamic and aspartic acids. The twelve diets were given to twenty-four growing pigs (30–40 kg) in three periods according to a randomized block design. Each period lasted 8 d and N retention was measured during the last 5 d of each period. Reducing dietary methionine, valine or isoleucine reduced the utilization of N (N retained/N digested) by approximately 20% ( $P < 0.05$ ). Adding leucine to the isoleucine-limiting diet decreased the utilization of N by 9% ( $P < 0.05$ ). This was reversed by simultaneous addition of valine. Excess leucine in a valine-deficient diet did not significantly reduce N utilization. In methionine-limiting diets an excess of either leucine alone or of all three BCAA increased the utilization of N by 8% ( $P < 0.05$ ).

### Branched-chain amino acids: Methionine: Amino acid utilization

Interactions amongst the branched-chain amino acids (BCAA) have been investigated mainly in rats (Harper *et al.* 1970, 1983; Block, 1989) and poultry (D'Mello & Lewis, 1970; Calvert *et al.* 1982). However, in most experiments relatively large excesses of BCAA were used. Leucine appears to be the most potent among the BCAA and excess dietary leucine has been shown to reduce the concentrations of valine, isoleucine and their  $\alpha$ -keto acids in plasma and tissues (Tannous *et al.* 1966; D'Mello & Lewis, 1970; Block & Harper, 1991) and to enhance the activity of branched-chain keto-acid dehydrogenase (EC 1.2.4.4; BCKDH) in various tissues (Frick *et al.* 1981; Block *et al.* 1985; Aftring *et al.* 1986). The stimulation of BCAA catabolism by dietary leucine excess could account for the depletion of the plasma pools of valine, isoleucine,  $\alpha$ -ketoisovaleric acid and  $\alpha$ -keto- $\beta$ -methyl-n-valeric acid, which appears to depress food intake and growth (Harper *et al.* 1983; Block, 1989). Studies by Calvert *et al.* (1982) in chicks and by Block & Harper (1984) in rats showed that a relatively large excess of leucine ( $>38$  g/kg diet) increased

the oxidation of isoleucine and valine. Increased oxidation would affect the utilization of these amino acids and might consequently affect animal performance when one of the two amino acids is limiting for growth. However, BCAA concentrations in practical diets do not normally exceed twice the requirement (15 g/kg); hence, from the practical point of view, it seems to be more important to investigate how moderate BCAA excesses could affect animal performance.

The importance of interactions among the BCAA in the pig is not well-established. Oestemer *et al.* (1973) found that increasing dietary leucine concentration from 7.0 to 10.2 g/kg diet in diets marginally deficient in isoleucine reduced the concentrations of valine and isoleucine in plasma. However, the effects on the efficiencies of food and protein conversion were not consistent. Henry *et al.* (1976), using a similar leucine excess, confirmed the effects on plasma valine and isoleucine concentrations but did not show any effect on growth performance.

An increase in dietary leucine from 13.4 to 20.4 g/kg in

**Abbreviations:** BCAA, branched-chain amino acids; BCKDH, branched-chain keto-acid dehydrogenase.

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diets marginally deficient in isoleucine (3.8 g/kg) reduced growth performance and plasma valine and isoleucine concentrations (Taylor *et al.* 1984). These authors concluded that this might have resulted from a reduction in isoleucine availability caused by the higher dietary leucine concentration.

The catabolism of methionine is linked with that of the BCAA via BCKDH (Benevenga, 1984). *In vitro* results from incubations of rat liver suggest that methionine decarboxylation is affected by branched-chain keto acids (Livesey, 1981). However, whether BCAA excesses affect methionine catabolism *in vivo* is not known.

The present experiment was conducted to investigate the importance and mechanism of amino acid interactions in the growing pig, especially the regulatory effects amongst the BCAA and methionine. The aims of the experiment were, first, to confirm that BCAA interactions affect protein utilization in pigs even at moderate levels of excess and, second, to see if, *in vivo*, methionine utilization is altered by moderate excesses of BCAA. These results were reported briefly to the Nutrition Society (Langer & Fuller, 1994).

## Materials and methods

### Animals

Twenty-four Cotswold crossbred gilts, 10 weeks of age, with an average starting weight of 29.6 (SD 2.62) kg were used in this experiment. Their mean finishing weight was 39.1 (SD 3.54) kg. All management and experimental procedures in this study were carried out in strict accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 by staff licensed under this Act to carry out such procedures.

### Diets and feeding

The pigs were given a low N intake to ensure that differences in response between balanced and unbalanced diets would be expressed. Four isoenergetic and isonitrogenous diets based on casein and synthetic amino acids were used (Tables 1 and 2), either with an optimally balanced amino acid pattern (Wang & Fuller, 1989, 1990; histidine content (2.5 g/16 g N) was taken from Wang & Fuller, 1990) or made limiting (80% of requirement) in methionine, valine or isoleucine. Each of these diets was given alone (treatments 1–4), or supplemented with leucine (50% excess; treatments 5–8), or supplemented with leucine (50% excess) together with the other non-limiting BCAA (treatments 9–12) (i.e. the valine-deficient diet had 50% excess isoleucine, the isoleucine-deficient diet had 50% excess

valine, and the control and methionine-deficient diets had 50% excess of each). This gave a total of twelve diets. Valine, isoleucine, leucine and methionine were added or removed at the expense of aspartic acid and monosodium glutamate.

The diets were given at the rate of 80 g/kg body weight<sup>0.75</sup> per d which was calculated to supply about 1.25 g N/kg body weight<sup>0.75</sup>. The animals received their diets twice daily at 08.30 and 15.30 hours. Water was freely available by nipple drinkers and was also added to the diets during feeding to reduce spillage.

### Experimental design

After a 7 d adaptation period on the optimally balanced control diet the twelve diets were given in three periods according to a randomized block design (Table 3). Each period lasted 8 d, made up of 3 d adjustment and 5 d collection. Each animal received three of the twelve diets and none of the animals had the same diet twice. Throughout the experiment the animals were kept individually in metabolism cages at a room temperature of 22–24°.

### Sample collection, storage and chemical analysis

During the last 4 d of each period urine was collected into 200 ml 2 M-H<sub>2</sub>SO<sub>4</sub> using bladder catheters introduced as described by Fuller *et al.* (1979). Two successive 24 h collections were pooled. Faeces were collected during the last 5 d of each period and preserved in 2 M-H<sub>2</sub>SO<sub>4</sub> (approximately 500 ml/d). After homogenization a portion was taken for analysis. Urine and faeces samples were stored at –20° and diet samples were stored at +4°. Diets, urine and faeces were analysed for N by the macro-Kjeldahl procedure (Davidson *et al.* 1970). Amino acid concentrations (except methionine, cyst(e)ine and tryptophan) were determined by ion-exchange chromatography. Concentrations of methionine, cyst(e)ine and tryptophan in the diets were analysed by Degussa AG (Hanau, Germany). Methionine and cyst(e)ine concentrations were determined after oxidation with performic acid as described by Bech-Andersen *et al.* (1990) and tryptophan concentration according to Naumann & Bassler (1993). Amino acid concentrations were calculated using the molecular mass of the free amino acid.

### Statistical analysis

A crossover design was used, with twelve treatments applied over three periods to twenty-four animals, each

**Table 1.** Experimental design\*

Limiting amino acid	Amino acid as % of requirement		
	No excess	Leucine	BCAA
None (control)	100 (T1)	150 (T5)	Leucine 150 + isoleucine 150 + valine 150 (T9)
Methionine	80 (T2)	150 (T6)	Leucine 150 + isoleucine 150 + valine 150 (T10)
Valine	80 (T3)	150 (T7)	Leucine 150 + isoleucine 150 (T11)
Isoleucine	80 (T4)	150 (T8)	Leucine 150 + valine 150 (T12)

BCAA, branched-chain amino acids; T, treatment.

\* For further details, see above.

**Table 2.** Ingredient composition and amino acid pattern of the control diet (air-dry basis)

Control diet		Concentration (g/16 g N)		
Ingredient	g/kg	Amino acid	Calculated	Measured
Casein*	59.00	Aspartic acid	26.77	26.78
Maize starch	323.85	Threonine	4.70	4.78
Glucose	238.67	Serine	2.82	3.41
Sugar	150.00	Glutamic acid	38.11	37.05
Powdered cellulose	65.00	Proline	6.28	6.54
Oil (vegetable)	40.00	Glycine	3.32	3.53
Mineral–vitamin mix†	53.13	Alanine	2.33	2.47
Aspartic acid	22.05	Valine	4.90	4.92
Threonine	2.00	Cyst(e)ine	1.64	1.58
Monosodium glutamate	28.05	Methionine	2.46	2.79
Alanine	0.70	Isoleucine	3.90	3.70
Glycine	2.20	Leucine	7.20	6.95
Valine	1.22	Tyrosine	3.90	3.85
Cyst(e)ine	1.39	Phenylalanine	3.90	3.92
Methionine	0.96	Lysine	6.50	6.61
Isoleucine	0.98	Histidine	2.50‡	2.68
Leucine	2.03	Arginine	4.30	4.13
Tyrosine	0.97	Tryptophan	1.20	1.05
Phenylalanine	1.05			
Lysine-HCl	2.62			
Histidine-HCl	1.20	Digestible energy (MJ/kg)	17.16	ND
Arginine-HCl	2.41	N (g/kg)	15.53	16.29
Tryptophan	0.53			

ND, not determined.

\* Casein contained: N (g/kg) 139.87; amino acids (g/16 g N): Asp 7.62, Thr 4.33, Ser 5.30, Glu 24.42, Pro 11.82, Gly 1.99, Ala 3.03, Val 6.83, Cys 0.35, Met 2.70, Ile 5.44, Leu 9.40, Tyr 5.41, Phe 5.20, Lys 8.20, His 2.76, Arg 4.15, Trp 1.20.

† Mineral and vitamin mixture contained (g/kg): Pigvite 12 (Norvite-Feed Supplements, Aberdeenshire, UK) 2.3, dicalcium phosphate (48%) 37, potassium bicarbonate 9.4, magnesium oxide 0.6, Vitamin B<sub>12</sub> Customix (Norvite-Feed Supplements) 1.4, choline chloride (50%) 2.1, and (mg/kg): iron sulfate 88.8, nicotinic acid 8.4, pteroylmonoglutamic acid 1.9, biotin (2%) 2.3, pantothenic acid 6.3, pyridoxine 2.5, thiamin 1.9, riboflavin 0.8, inositol 138.8, ascorbic acid 11.1.

‡ From Wang & Fuller (1990).

animal receiving three treatments and each treatment being allocated to two animals in any one period. Groups of four similar animals (blocks) were chosen to form complete replicates, there being six of these. The allocation of treatments to animals over three periods is given in Table 3. The data were analysed using a generalized linear model (GENSTAT 5.2; Rothamsted Experimental Station, Harpenden, Herts., UK) with terms for animal, treatment and period plus a covariate for N intake ( $23 + 11 + 2 + 1 = 37$  d.f.). No allowance was made for carry-over effects and there was no recovery of 'inter-block' information.

### Results

All animals grew satisfactorily (although slowly as a consequence of the low-N diet) and there were only a few food refusals. These were dried and food intake and N intake were corrected on a DM basis. Two animals had blocked urinary catheters in period 1, but recovered satisfactorily to continue the experiment. Data from these two animals (for period 1 only) were excluded from the analysis. The calculated amino acid pattern of all diets was confirmed by amino acid analysis. The pattern in the control diet is given in Table 2.

N retention was calculated as N intake – (urinary N + faecal N); N utilization (Table 4) was expressed as N retained : N digested. N digested was calculated as N intake – faecal N.

**Table 3.** Allocation of treatments (T1–T12) to animals (A1–A24) over three periods (P1–P3)

Animal	Period		
	P1	P2	P3
A1	T1	T5	T9
A2	T2	T6	T10
A3	T3	T7	T11
A4	T4	T8	T12
A5	T5	T10	T4
A6	T6	T11	T1
A7	T7	T12	T2
A8	T8	T9	T3
A9	T9	T4	T6
A10	T10	T1	T7
A11	T11	T2	T8
A12	T12	T3	T5
A13	T1	T6	T12
A14	T2	T9	T9
A15	T3	T8	T10
A16	T4	T5	T11
A17	T5	T11	T2
A18	T6	T12	T3
A19	T7	T9	T4
A20	T8	T10	T1
A21	T9	T3	T5
A22	T10	T4	T6
A23	T11	T1	T7
A24	T12	T2	T8

**Table 4.** Influence of leucine or branched-chain amino acid (BCAA) excesses on nitrogen utilization (nitrogen retained:nitrogen digested\*) in diets limiting in methionine, isoleucine or valine (Mean values with their standard errors for six pigs)

Limiting amino acid	Amino acid excess					
	No excess		Leucine		BCAA†	
	Mean	SE	Mean	SE	Mean	SE
None	0.77 <sup>a</sup>	0.015	0.75 <sup>a</sup>	0.015	0.76 <sup>a</sup>	0.014
Methionine	0.67 <sup>b</sup>	0.014	0.72 <sup>ab</sup>	0.016	0.72 <sup>ab</sup>	0.016
Valine	0.68 <sup>b</sup>	0.014	0.66 <sup>b</sup>	0.015	0.70 <sup>b</sup>	0.017
Isoleucine	0.67 <sup>b</sup>	0.016	0.61 <sup>c</sup>	0.014	0.66 <sup>b</sup>	0.014

<sup>a,b,c</sup> Mean values within a row or column not sharing a common superscript were significantly different ( $P < 0.05$ ).

\* N retained = N intake - (urinary N + faecal N); N digested = N intake - faecal N.

† See Table 1.

Compared with the control diet N utilization was significantly ( $P < 0.05$ ) and almost equally decreased by the 20% reductions of isoleucine, valine or methionine (Table 4). Additions of leucine or BCAA to the balanced control diet had no significant effect on N utilization. Adding leucine to an isoleucine-limiting diet decreased N utilization significantly ( $P < 0.05$ ). This was reversed by the simultaneous addition of valine. Addition of leucine to the valine-limiting diet tended to reduce N retention and utilization, a tendency which was reversed by the simultaneous addition of isoleucine: however, these effects were not significant.

In contrast, adding either leucine alone or all three BCAA to a methionine-limiting diet, significantly ( $P < 0.05$ ) increased N utilization.

## Discussion

N utilization was similarly reduced by the 20% reductions in the dietary concentrations of valine, isoleucine or methionine. Therefore, it can be concluded that these diets were equally deficient in their specific limiting amino acid. Consequently any change in N utilization should reflect a change in utilization of the limiting amino acid.

### Valine- and isoleucine-deficient diets

Because the BCAA interact with each other the reduction in utilization of N due to excess leucine in a diet limiting in isoleucine was to be expected. This observation may be explained if significant amounts of isoleucine are lost via catabolic processes activated by excess leucine. Assuming that leucine excess increases the catabolism of valine and isoleucine in the pig in the same way as in chicks, rats and human subjects (Calvert *et al.* 1982; Meguid *et al.* 1983; Block & Harper, 1984), increased catabolism of valine or isoleucine would have reduced the utilization of these amino acids resulting in reduced utilization of dietary N.

The present findings show that isoleucine-limiting diets were more affected by an excess of leucine than valine-limiting diets, indicating that leucine affected the utilization of isoleucine more than that of valine. Benton *et al.* (1956) came to a similar conclusion using growing rats. In contrast, results of an experiment with young chicks suggest that the

leucine–valine interaction seems to be more potent than the interaction between leucine and isoleucine (D'Mello & Lewis, 1970). The reason why the two species responded differently is not known.

The observation that, with isoleucine-deficient diets, addition of valine reversed the effect of excess leucine can be explained by competition between their keto acids for decarboxylation via BCKDH. In the rat it has been shown that  $\alpha$ -ketoisovaleric and  $\alpha$ -keto- $\beta$ -methyl-n-valeric acids have similar  $K_m$  values (Parker & Randle, 1978; Boyer & Odessey, 1990) and compete as substrates for BCKDH. Hence, when an alternative substrate is provided, competition between the keto acid of the amino acid in excess and the keto acid of the limiting amino acid may reduce the oxidation of the limiting amino acid and lead to increased N utilization.

### Methionine-deficient diets

Somewhat surprisingly, excess leucine as well as excess BCAA enhanced N retention in pigs given diets limiting in methionine. It appears that excesses of BCAA in methionine-limiting diets have the effect of sparing methionine. The increased protein accretion could be the result of either increased protein synthesis or decreased protein degradation or both. Leucine and  $\alpha$ -ketoisocaproic acid have been shown *in vitro* to increase protein synthesis in rat muscle (Fulks *et al.* 1975; Li & Jefferson, 1978) and also appear to reduce muscle protein degradation (Goldberg & Tischler, 1981). Although there is *in vitro* evidence that leucine alters protein synthesis, the *in vivo* effect, with amino-acid-adequate diets, is questionable (McNurlan *et al.* 1982, 1983).

Another possible way in which leucine or BCAA might interact with methionine catabolism is through the BCKDH complex which catabolizes branched-chain keto acids and also  $\alpha$ -ketobutyric acid and  $\alpha$ -keto- $\gamma$ -methylbutyric acid, keto acids produced from methionine. Benevenga & Haas (1986) reported that the pig is capable of oxidizing methionine via the transamination pathway in liver mitochondria. In the rat, excess leucine or other BCAA seems to increase the catabolism of their keto acids (Block & Harper, 1984; Block *et al.* 1985) which would lead to a greater rather than lower oxidation of methionine. However, it may be that the provision by the amino acid in excess of an alternative substrate at a higher concentration actually diminishes the oxidation of the limiting amino acid. It is not known whether BCKDH in pig tissues can be activated by excesses of leucine or BCAA, or whether leucine excess alters keto acid concentrations in pigs to the same extent as in rats. It also seems necessary to consider whether methionine is catabolized via the transamination pathway in the pig under the conditions of the present experiment when methionine was limiting. These issues are examined in the following paper (Langer *et al.* 2000).

### Non-deficient diets

Unlike their effects with the methionine-limiting diet, excesses of leucine or BCAA added to the balanced control diet did not significantly alter N retention. As there would be

competition for intestinal transport amongst the BCAA with the balanced diets, as with the imbalanced ones, this seems to support the suggestion that in post-absorptive metabolism these interactions exert their effects on N retention. Thus, under these conditions, moderate excesses of BCAA appeared to have little, if any, effect on the utilization of other amino acids. However, because the control diet was designed to be ideally balanced, it is also possible that a potential positive effect could have been limited by one or more of the other indispensable amino acids. When methionine was limiting all other indispensable amino acids were in relative excess and any increase in methionine utilization could be expressed as increased body protein accretion but when, in the control diet, all amino acids were equally limiting, improvement in the utilization of any one would not result in increased N retention unless there was a simultaneous improvement in the utilization of all the others.

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