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Hyperinsulinaemia, supplemental protein and branched-chain amino acids when combined can increase milk protein yield in lactating sows

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The aim of this study was to determine whether dietary supplementation with branched-chain amino acids, and the infusion of insulin and dextrose, would increase milk protein secretion in the sow. The experiment involved sixteen lactating sows fed either a normal lactation diet (162 g/kg crude protein, *n* 8) or a high-protein diet (230 g/kg crude protein, *n* 8) supplemented with branched-chain amino acids (valine, isoleucine and leucine). Sows were either infused with insulin and dextrose or not infused at all during mid (day 5–10) and late (day 17–22) lactation in a single reversal design. Blood samples were analysed for glucose, and the dextrose infusion rate was adjusted to maintain the blood glucose level within 15 % of pre-infusion levels. Milk (10·1 *v.* 11·1 kg/d; $P=0{\cdot}014$) and lactose (628 *v.* 727 g/d; $P=0{\cdot}002$) yield increased with insulin infusion, whereas milk protein content (5·0 % *v.* 5·5 %; $P=0{\cdot}007$) was increased in diets supplemented with protein and branched-chain amino acids. Piglet growth was increased by feeding the higher-protein diet (237 *v.* 273 g/d; $P=0{\cdot}05$) but not significantly increased by insulin infusion (245 *v.* 265 g/d; $P=0{\cdot}11$). These effects were additive such that the combined treatment resulted in a 24 % (56 g/d; $P<0{\cdot}05$) increase in piglet growth rate. These data demonstrate that increasing the dietary protein/branched-chain amino acid content can increase milk protein secretion but not milk yield. The infusion of insulin and dextrose increased milk and milk lactose yields, and tended to increase milk protein yield but not milk protein content. These effects are additive and translate to increased protein yield and piglet growth.

Pig: Lactation: Insulin: Branched-chain amino acids: Protein

The pig has evolved to have a high fat content in its milk compared with that of other domesticated animals, to aid the survival of piglets born with very little insulation or energy reserves (Mellor & Cockburn, 1986). Today, however, pigs are kept in more controlled settings and do not require milk with such a high fat (relative to protein) content after the first few days of life. Indeed, such a milk may be a serious constraint to piglet growth performance. The protein requirement of artificially reared pigs of between 2 and 7 kg is 10·7–11·5 g/MJ (Williams, 1976; Auldist *et al.* 1997), whereas the protein content of sow's milk is less than 8·0 g/MJ (King *et al.* 1993*b*). Therefore, if the milk protein content of sow's milk can be increased without necessarily changing milk yield, sucking pig growth can be increased by up to 40 %. This is particularly important during late lactation when milk yield is generally insufficient to maintain piglet growth rate (Dunshea, 2003). For example, sow milk production increases over the first 2 weeks of lactation before reaching a plateau (Toner *et al.* 1996), and thus the extent to which milk yield limits piglet growth rate is exacerbated as lactation advances (Dunshea, 2003).

McGuire *et al.* (1995) demonstrated that the simultaneous infusion of insulin and dextrose increased milk protein content and yield by 7 % and 3·5 %, respectively, in dairy cows. Interestingly, the circulating concentrations of amino acids, in particular the branched-chain amino acids (BCAA), were markedly decreased, presumably because they were preferentially taken up and utilised by the mammary gland through either oxidation or incorporation into milk proteins. In a subsequent study in which additional protein was supplied as an abomasal infusion of casein, it was found that whereas casein and insulin/dextrose increased milk protein yield by 10 % and 4 %, respectively, the combined treatment increased milk protein yield by 28 % (Griinari *et al.* 1997). In this context, the amino acid profile and ileal digestibility of casein, including the BCAA, is similar to that of whole-milk protein (Rutherfurd & Moughan, 1998), whereas the relative proportions of BCAA are in excess of those in muscle and other tissues (Reeds & Mersmann, 1991). Despite supplying additional protein as casein, the infusion of insulin and dextrose decreased the plasma concentrations of the BCAA valine, isoleucine and leucine by approximately 60 % while the plasma concentrations of the other essential amino acids decreased by an average of 25 % (range 13–40 %). It is therefore possible that supplying additional BCAA may stimulate milk protein yield even further if the depression in BCAA can be avoided. The following study was thus conducted to determine whether insulin and dextrose infusion, and supplemental protein and BCAA, could increase milk yield and piglet growth.

Abbreviations: BCAA, branched-chain amino acids; CP, crude protein; NEFA, non-esterified fatty acids; FCR, feed conversion ratio.

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Materials and methods

Experimental design and methodology

All procedures involving animals were approved by the Victorian Institute of Animal Science Animal Ethics Committee. Sixteen cross-bred (Large White × Landrace) multiparous sows nursing litters of eleven piglets were maintained in farrowing crates throughout the study. Sows were catheterised on the morning of day 4 of lactation after having their feed removed for 12 h. Muscle relaxation was induced with an intramuscular injection of azoperone (Stresnil, 40 mg/ml; Boehringer, North Ryde, New South Wales, Australia), after which anaesthesia was induced and maintained with halothane (Fluothane; Orica, Ascot Vale, Victoria, Australia). Two silastic catheters (Sil-Med Corporation, Taunton MA, USA; 0·16 mm internal diameter, 0·32 mm external diameter) were inserted 15 cm into the anterior vena cava via each of the cephalic veins (Dunshea & King, 1995). The catheters were exteriorised in the region of the interscapular space on the back of the animals and stored in a cloth pocket glued to the back. After catheterisation, sows were given a 4 d course of broad-spectrum antibiotic (Terramycin/LA, oxytetracycline 200 mg/ml; Pfizer, West Ryde, New South Wales, Australia) and exit wounds were treated with antibiotic powder (Terramycin, oxytetracycline, 20 mg/g; Pfizer). Catheters were flushed daily with physiological saline containing K_2EDTA (12·5 g/l).

Sows were randomly allocated to either a conventional lactation ration (162 g/kg crude protein (CP)) or a high-protein diet (230 g/kg CP) with supplemental free BCAA (valine, isoleucine and leucine) for the duration of lactation (Table 1). Sows were offered 3·0 kg of their respective diet on the first day of lactation, and the daily allocation was increased by 0·5 kg increments until *ad libitum* intakes were achieved. Commencing on day 10 of lactation, half the sows were bled at 08.00 h every day for 5 d, and the blood was rapidly analysed with a blood glucose monitor to determine blood glucose concentrations. The other half of the sows were bled every 30 min for 2 h from 08.00 h, and the blood was rapidly analysed with a blood glucose monitor to determine baseline blood glucose concentrations.

Table 1. Experimental diets (g/kg)

	Control	High protein/BCAA
Ingredient		
Wheat (11 % CP)	635·5	544·3
Wheat bran	100·0	100·0
Soyabean meal (48 % CP)	91·0	81·1
Fish meal (65 % CP)	30·7	50·0
Blood meal (83 % CP)	6·3	40·0
Tallow	29·0	14·8
Limestone	6·8	10·0
Dicalcium phosphate	17·3	19·1
Salt	2·0	2·0
Vitamins and mineral premix	2·0	2·0
Skim milk powder	70·0	120·0
Lysine	4·57	1·63
Methionine	1·78	2·13
Threonine	1·78	0·73
Valine	1·38	5·28
Isoleucine		3·45
Leucine		3·42
Estimated composition		
Digestible energy (MJ/kg)	14·5	14·5
Crude protein	162	230
Available lysine	9·6	12·8
Available valine	7·70	15·5
Available isoleucine	5·30	10·6
Available leucine	9·80	20·0
Total available BCAA:lysine (g:g)	2·37	3·60

BCAA, branched-chain amino acid; CP, crude protein.

Commencing at 10.00 h on day 10, the latter half of the sows were infused with insulin (11 mU/kg per h) and sufficient dextrose (50 % dextrose) to maintain euglycyaemia for 5 d. The dose of insulin was chosen as the maximum dose that did not reduce feed intake in a preliminary study (see discussion). To achieve euglycaemic conditions, venous blood samples were obtained every 2 h for the first 2 d and rapidly analysed for glucose concentrations, and the dextrose infusion rate was adjusted to maintain blood glucose within 15 % of the pre-infusion levels. After 2 d, blood glucose was measured at least every 8 h, and infusion rates were adjusted only if glycaemia fluctuated dramatically. Milk yield was determined by ^2H_2O dilution between days 1 and 5 of infusion (Prawirodigdo *et al.* 1990) and milk samples were obtained on days 3 and 5 to determine milk composition.

After nursing at approximately 09.00 h on day 1, the piglets were separated from the dam for 45 min to standardise gut fill and were weighed. Each piglet was then injected with a weighed dose of ^2H_2O (2·0 g/kg live weight). At 1 h after injection, a 5 ml blood sample was obtained via vena cava puncture in order to determine the equlibration concentration of ^2H_2O in the body fluid. After nursing at approximately 09.00 h on day 5 of the treatment period, the pigs were separated from the dam for 45 min and another 5 ml blood sample was obtained. The concentration of ^2H_2O in the plasma was determined using Fourier transform-infrared spectrometry, as described by Glencross *et al.* (1997). Since milk was the only source of water consumed by the piglets, the milk yieid of the sows was calculated from the water turnover of the piglets, as described previously (Prawirodigdo *et al.* 1990; King *et al.* 1993*b*).

Milk samples (5–10 ml per gland) were collected from three medial glands during a suckling bout intitiated by the piglets (i.e. without the use of oxytocin). Plasma samples were obtained for insulin, glucose, amino acids and non-esterified fatty acids (NEFA). Infusions commenced on day 10 and continued until day 15. Sows that had not received the hyperinsulinaemic–euglycaemic clamp earlier received the insulin/dextrose infusion from day 17 to 22, and milk yield and composition were determined as above. Milk yield was determined as before, with the exception that a blood sample was taken before ^2H_2O injection to determine residual ^2H_2O.

Chemical analyses

During the hyperinsulinaemic–euglycaemic clamp, blood glucose concentrations were monitored using a blood glucose monitor and strips (Accucheck; Roche, Mannheim, Germany). Plasma was analysed for insulin, glucose and NEFA using kit assays validated in our laboratory (Ostrowska *et al.* 2002). Inter- and intra-assay variations were 9·2 % and 6·1 %, 2·9 % and 1·6 %, and 5·8 % and 4·8 % for insulin, glucose and NEFA, respectively. Plasma and milk amino acids were analysed by ion-exchange chromatography (Rayner, 1985; King *et al.* 1993*a*). Milk samples were prepared for amino acid analyses by freeze-drying, followed by fat extraction with a mixture of chloroform and acetate. The fat-free sample was air-dried and ground using a mortar and pestle, and then passed through a 0·5 mm sieve prior to analyses (King

et al. 1993*b*). Milk protein, lactose and fat were analysed in whole liquid milk as described by Atwood & Hartmann (1992). Ash was assumed to be 8·8 g/kg, and total solids were estimated by the sum of ash, protein, lactose and fat (King *et al.* 1993*b*).

Statistics

Growth and plasma constituent data were analysed by ANOVA, with the main effects being insulin infusion, stage of lactation and diet. Blocking structure was sow. Growth rate was measured between days 1 and 5 of each infusion, whereas the ANOVA for blood and plasma constituents was conducted using the pooled average of the samples obtained at 08.00 h on each day for each sow.

Results

Basal blood glucose was not affected by dietary protein content (4·04 *v.* 3·99 mmol/l; $P=0·68$), and the simultaneous infusion of dextrose ensured that euglycaemia was maintained during the insulin infusion (4·02 *v.* 4·01 mmol/l; $P=0·97$; Fig. 1). There was no effect of dietary protein on basal insulin concentrations (27·2 *v.* 24·5 mU/l; $P=0·52$), whereas insulin infusion at a rate of 11 mU/kg per h increased pre-feeding plasma insulin concentrations by approximately 50 % (21·1 *v.* 31·4 mU/l; $P=0·018$; Fig. 2). The amount of dextrose required to maintain euglycaemia was lower in sows fed the high-protein diet supplemented with BCAA (3·91 *v.* 2·80 mmol/min; $P<0·001$; Fig. 1) and was higher during mid compared with late lactation (2·94 *v.* 3·76 mmol/min; $P=0·005$). There was, however, a significant interaction ($P<0·001$) such that the amount of dextrose infused was lower in sows fed the high-protein diet supplemented with BCAA during mid lactation (4·15 *v.* 2·06 mmol/min) but not during late lactation (3·67 *v.* 3·85 mmol/min).

Piglet growth was increased by feeding the higher-protein diet supplemented with BCAA (237 *v.* 273 g/d; $P=0·051$) but not significantly increased by insulin infusion (245 *v.* 265 g/d; $P=0·11$). The effects were, however, additive such that the combination of the high-protein + BCAA diet and hyperinsulinaemia resulted in a 24 % (229 *v.* 285 g/d; $P<0·01$) increase in piglet growth rate (Fig. 3). Litter growth was increased by feeding the higher-protein diet (2·60 *v.* 2·91 kg/d; $P=0·044$) but not significantly increased by insulin infusion (2·66 *v.* 2·85 kg/d; $P=0·15$). Again, however, effects were additive such that the combined treatment resulted in a 20 % (2·52 *v.* 3·03 kg/d; $P<0·01$) increase in litter growth rate.

Milk yield was not altered by dietary protein (10·7 *v.* 10·5 kg/d; $P=0·92$), whereas it was increased during insulin infusion (10·1 *v.* 11·1 kg/d; $P=0·014$; Table 2). Milk protein content was increased by feeding additional protein and BCAA (5·0 *v.* 5·5 %; $P=0·007$) but was not altered during insulin infusion (5·3 *v.* 5·1 %; $P=0·29$). Milk protein output was not significantly changed by dietary protein and BCAA (530 *v.* 574 g/d; $P=0·24$), although an increase of similar magnitude during insulin infusion approached statistical significance (534 *v.* 571 g/d; $P=0·099$). Effects were additive such that the combined treatment resulted in a 16 % (523 *v.* 605 g/d; $P<0·01$) increase in milk protein output.

Milk fat content was neither changed by dietary protein (6·9 *v.* 6·8 %; $P=0·91$) nor significantly altered during the insulin infusion (7·3 *v.* 6·3 %; $P=0·13$). Milk fat output was not altered by dietary protein (732 *v.* 718 g/d; $P=0·89$) or insulin infusion (738 *v.* 711 g/d; $P=0·75$). Milk lactose content was neither changed by dietary protein (6·4 *v.* 6·4 %; $P=0·80$) nor significantly altered during insulin infusion (6·3 *v.* 6·5 %; $P=0·14$). Milk lactose output was not altered by dietary protein (684 *v.* 671 g/d; $P=0·81$) but was markedly increased during insulin infusion (628 *v.* 727 g/d; $P=0·002$) due to a combination of both increased milk yield and a non-significant increase in milk lactose content. The ratio of protein:energy in milk was not significantly increased by dietary protein (10·2 *v.* 10·9 g/MJ gross energy; $P=0·11$) and was unchanged during insulin infusion (10·4 *v.* 10·7 g/MJ gross energy; $P=0·57$).

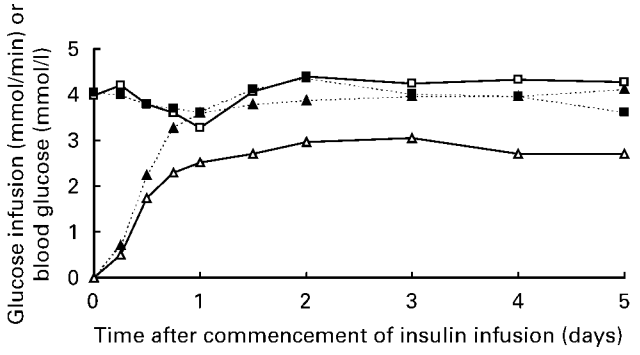

Fig. 1. Effect of basal (closed symbols) or high-protein + branched-chain amino acids (open symbols) on blood glucose (□, ■) or the dextrose infusion rate (△, ▲) required to maintain glycaemia during insulin infusion (11 mU/kg per h) in sows. SED (for diet × insulin × day) for glucose infusion rate and blood glucose was 1·2 mmol/min and 0·46 mmol/l, respectively.

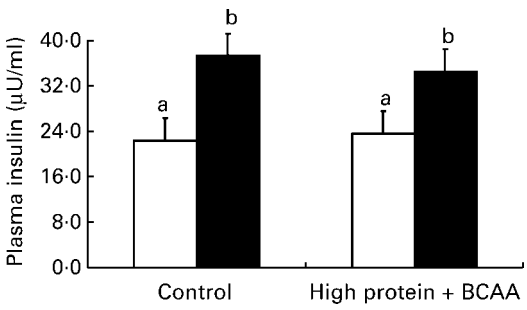

Fig. 2. Effect of control or high-protein + branched-chain amino acid (BCAA) lactation diets under basal conditions (□) gross energy or during a simultaneous insulin and glucose infusion (■) on plasma insulin. Values are means and SED for diet × insulin. Values with different letters are significantly different ($P<0·05$).

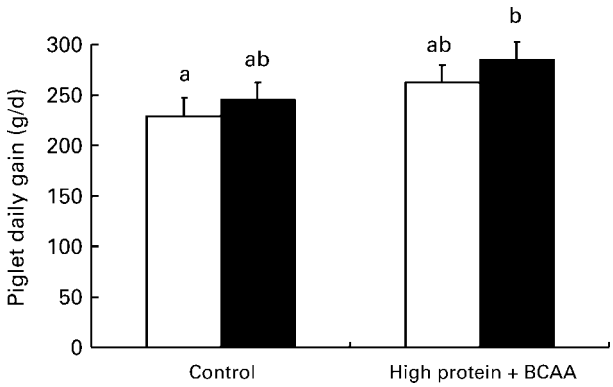

Fig. 3. Effect of control or high protein + branched-chain amino acid (BCAA) lactation diets under basal conditions (□) or during a simultaneous insulin and glucose infusion (■) on piglet daily gain. Values are means and SED for diet × insulin. Values with different letters are significantly different ($P<0·05$).

Table 2. Effect of dietary protein, insulin infusion and stage of lactation on milk yield and macronutrient composition and piglet feed conversion ratio (FCR)*

Diet (D)	Control				High protein/BCAA					Significance		
Insulin (I)	Basal		Insulin		Basal		Insulin		SED†			
Lactation stage (S)	Mid	Late	Mid	Late	Mid	Late	Mid	Late		D	I	S
Piglet growth (g/d)	222	237	241	247	287	236	266	303	29·4	0·051	0·11	0·95
Litter growth (g/d)	2486	2552	2565	2775	2927	2653	2970	3088	303·0	0·044	0·15	0·83
Milk yield (g/pig per d)	930	951	947	1069	978	851	943	1155	113	0·92	0·016	0·14
Milk yield (g/litter per d)	10 472	10 203	10 090	12 015	9982	9552	10 579	11 767	1165	0·77	0·014	0·13
Milk protein (%)	5·48	4·75	4·93	4·83	5·65	5·50	5·40	5·42	0·34	0·007	0·29	0·21
Milk protein (g/d)	564	483	494	580	564	524	574	635	59	0·24	0·10	0·76
Milk fat (%)	7·15	7·74	6·73	5·78	6·94	7·42	5·18	7·56	1·23	0·91	0·13	0·32
Milk fat (g/d)	750	801	681	696	694	709	555	914	181	0·89	0·75	0·20
Milk lactose (%)	6·31	6·22	6·44	6·57	6·35	6·18	6·87	6·26	0·30	0·80	0·14	0·30
Milk lactose (g/d)	660	630	651	794	636	587	729	733	169	0·81	0·002	0·51
Protein:energy (g/MJ)	10·7	9·5	10·0	10·6	11·0	10·4	12·0	10·2	1·0	0·11	0·57	0·21
FCR (g liquid/g)	4·24	4·00	3·94	4·33	3·41	3·60	3·57	3·81	0·27	0·004	0·42	0·23
FCR, (g solids/g)	0·83	0·78	0·75	0·79	0·68	0·72	0·66	0·77	0·07	0·037	0·70	0·28

BCAA, branched-chain amino acid.
*For details of diets and procedures, see Table 1 and text.
† SED for diet × insulin × stage of lactation. There were no significant ($P>0·05$) interactions.

The feed conversion rate (FCR) of liquid sow milk decreased by feeding supplemental protein (4·13 *v.* 3·60; $P=0·004$) but was unchanged during insulin infusion (3·81 *v.* 3·91; $P=0·42$). Likewise, the FCR of sow milk solids decreased by feeding supplemental protein (0·79 *v.* 0·71; $P=0·037$) but was unchanged during insulin infusion (0·75 *v.* 0·74; $P=0·70$). There were no major differences in macronutrient content or yields or the FCR of milk between mid and late lactation (Table 2).

The effects of dietary protein combined with supplemental BCAA and the hyperinsulinaemic–euglycaemic clamp on the amino acid composition of sow's milk are shown in Table 3. Increasing the dietary protein, combined with BCAA fortification, increased the content of many of the amino acids in milk, including alanine, aspartic acid, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine and valine. On the other hand, the milk content of arginine, glutamic acid, glycine and proline was relatively unaffected by dietary protein. Insulin and glucose infusion failed to alter the amino acid content of sow's milk (Table 3).

The output of valine in sow milk tended to be increased by feeding the higher-protein diet (30·8 *v.* 38·3 g/d; $P=0·089$) and by insulin infusion (31·6 *v.* 37·5; $P=0·060$). These effects were additive such that the combined treatment resulted in a 48 % increase in milk valine output (Fig. 4). The output of leucine in sow's milk tended to be increased by feeding the higher-protein diet (61·8 *v.* 71·1 g/d; $P=0·10$) and by insulin infusion (62·5 *v.* 70·4 g/d; $P=0·054$). These effects were additive such that the combined treatment resulted in a 30 % increase in milk leucine output (Fig. 4). The responses to the other BCAA, isoleucine, were qualitatively similar, although the differences were not as great and were not significant ($P>0·10$; Fig. 4).

Sow feed intake was not altered by dietary protein (6·4 *v.* 6·7 kg/d, $P=0·68$) or insulin infusion (6·8 *v.* 6·3 kg, $P=0·37$) but tended to be higher during late lactation (7·0 *v.* 6·1 kg, $P=0·089$). There was no effect of diet on plasma NEFA concentrations (261 *v.* 242 μmol/l; $P=0·61$), whereas insulin infusion decreased plasma NEFA (330 *v.* 173 μmol/l; $P<0·001$). There was, however, an interaction ($P=0·018$) between diet and insulin infusion such that plasma NEFA concentrations were higher and fell further during hyperinsulinaemia in sows eating the conventional diet (380 *v.* 142 μmol/l) compared with sows consuming the high-protein diet and supplemented BCAA diet (281 *v.* 204 μmol/l). The stage of lactation had no effect on plasma NEFA concentrations ($P=0·64$).

Increasing the dietary protein combined with BCAA fortification increased the plasma amino acid concentration of many amino acids, including alanine, arginine, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, tyrosine and valine (Table 4). On the other hand, the plasma concentrations of aspartic acid, citrulline, histidine, serine, taurine and threonine were relatively unaffected by dietary protein. Insulin and glucose infusion decreased the plasma concentrations of a few key amino acids, including isoleucine (−6 %), leucine (−10 %), methionine (−11 %), threonine (−9 %) and tyrosine (−11 %), although the magnitude of these decreases was relatively small (Table 4). The plasma concentrations of all amino acids were higher during late lactation compared with mid lactation, most differences being significant ($P<0·05$). There were also a number of two- and three-way interactions, although the magnitude of these was generally small.

Table 3. Effect of dietary protein, insulin infusion and stage of lactation on milk amino acid concentrations (g/kg)*

Diet (D)	Control				High protein/BCAA							
Insulin (I)	Basal		Insulin		Basal		Insulin			Significance		
Lactation stage (S)	Mid	Late	Mid	Late	Mid	Late	Mid	Late	SED†	D	I	S
Alanine	2·14	1·61	1·99	1·85	2·33	2·03	2·20	2·21	0·24	0·023	0·79	0·084
Arginine	2·94	2·32	2·51	2·42	2·96	2·54	2·75	2·78	0·34	0·22	0·70	0·16
Aspartic acid	6·30	4·34	5·43	5·07	6·62	5·60	6·13	6·28	0·83	0·031	0·98	0·11
Glutamic acid	15·3	13·7	15·3	14·2	15·3	14·7	15·3	14·8	0·93	0·40	0·75	0·080
Glycine	2·03	1·73	1·91	1·69	1·98	1·71	1·71	1·94	0·20	0·95	0·68	0·23
Histidine	1·52	1·11	1·43	1·22	1·48	1·33	1·53	1·40	0·18	0·21	0·71	0·036
Isoleucine	2·70	2·02	2·51	2·26	2·83	2·55	2·77	2·68	0·30	0·018	0·87	0·079
Leucine	6·53	4·78	6·18	5·71	7·03	6·51	6·99	6·62	0·63	0·002	0·66	0·057
Lysine	5·65	3·76	5·05	4·53	5·91	5·28	5·71	5·48	0·71	0·022	0·91	0·056
Methionine	1·16	0·82	1·06	0·95	1·17	1·05	1·15	1·08	0·24	0·066	0·85	0·024
Phenylalanine	2·45	1·85	2·33	2·05	2·52	2·26	2·44	2·43	0·26	0·054	0·76	0·068
Proline	6·11	5·72	6·98	5·45	6·35	5·76	5·95	6·18	0·60	0·99	0·62	0·093
Serine‡	4·19	2·75	3·40	3·12	4·25	3·49	3·75	4·11	0·56	0·045	0·81	0·12
Threonine‡	2·72	1·93	2·37	2·25	2·78	2·43	2·62	2·66	0·56	0·016	0·96	0·077
Tyrosine	2·49	1·93	2·35	2·11	2·61	2·44	2·59	2·40	0·28	0·029	0·97	0·10
Valine	2·60	2·63	3·30	2·98	3·91	3·39	3·72	3·60	0·55	0·013	0·36	0·43

BCAA, branched-chain amino acid.
* For details of diets and procedures, see Table 1 and text.
† SED for diet × insulin × stage of lactation. There were no significant ($P>0·05$) interactions except where indicated.
‡ Significant I × S interaction ($P<0·05$).

Discussion

These data demonstrate that the simultaneous infusion of insulin and dextrose increased milk output but not milk protein content. Consequently, the simultaneous infusion of insulin and dextrose tended to increase milk protein yield, particularly during late lactation. As a result, there was an increase in piglet growth rate, primarily due to increased milk intake since there was no effect on the efficiency with which milk from sows infused with insulin and dextrose was used for growth by the piglets. It has generally been held that the ruminant, and indeed the sow, mammary gland is insensitive to insulin, particularly with respect to glucose uptake and lactose production (Hove, 1978; Laarveld *et al.* 1981, 1985). However, the studies on which this has been based have been relatively short in duration (hours), and euglycaemia has generally not been maintained. To partially address this, Mackle *et al.* (2000*b*) conducted 4 d intramammary infusions of insulin that resulted in elevated milk insulin concentrations (25-fold) with no effect on systemic blood glucose concentrations. However, the intramammary insulin infusion had no effect on milk production or on milk fat and protein yields, further supporting the hypothesis that insulin does not appear directly to stimulate milk production or milk protein synthesis.

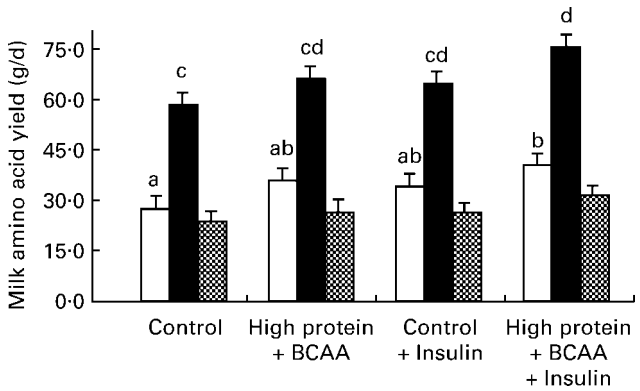

Fig. 4. Effect of control or high-protein + branched-chain amino acid (BCAA) lactation diets under basal conditions or during a simultaneous insulin and glucose infusion (Insulin) on milk BCAA yield. Values are means and SED for diet × insulin. Values within an amino acid with different letters are significantly different ($P<0·05$). □, Valine; ■, Leucine; ▦, Isoleucine.

Increasing the protein level from 162 to 230 g/kg while fortifying the diet with additional BCAA increased milk protein content and improved piglet growth. As a consequence, the milk from sows fed the higher-protein plus BCAA diet was used more efficiently than milk from sows fed what is generally considered to be a protein-adequate diet. These improvements in FCR presumably occur because of the increased protein:energy ratio in the milk from sows fed the fortified diets since increasing the dietary protein and BCAA level had no effect on milk yield. Whereas milk and protein yield can themselves increase with increasing lysine (protein) in the diet, particularly if energy intake is maximised (Tokach *et al.* 1992), the protein composition of milk still limits potential growth by 40 %. For example, King *et al.* (1993*b*) fed gilts diets ranging in protein content from 63 to 238 g/kg CP and found that only at the lower extremities were there any difference in the protein content of the milk. Certainly, increasing the dietary protein content above that fed in conventional rations (168 g/kg) had no effect on milk protein content (King *et al.* 1993*b*). The major difference between our study and that of King *et al.* (1993*b*) was that we added supplemental BCAA. It may therefore be possible that BCAA limit milk production in conventional diets fed to lactating sows even when additional protein is provided above current recommendations.

Richert *et al.* (1996, 1997) have shown that increasing the valine and isoleucine content of sow diets with relatively low protein contents (approximately 145 g/kg CP) will increase piglet litter growth. These authors also reported that increasing dietary isoleucine but not valine increased sow milk protein content (Richert *et al.* 1997). Interestingly, they also observed that increasing dietary valine, isoleucine and total BCAA resulted in

Table 4. Effect of dietary protein, insulin infusion and stage of lactation on plasma amino acids (μg/ml)*

Diet (D)	Control				High protein/BCAA							
Insulin (I)	Basal		Insulin		Basal		Insulin			Significance		
Lactation stage (S)	Mid	Late	Mid	Late	Mid	Late	Mid	Late	SED†	D	I	S
Alanine**	79·1	93·4	85·2	94·4	60·5	46·7	41·5	60·0	13·5	<0·001	0·91	0·021
Arginine‡	12·4	10·3	8·5	16·5	17·7	15·0	11·6	18·4	2·45	0·024	0·86	0·003
Aspartic acid**	6·0	10·1	9·4	6·7	7·2	7·2	5·8	7·3	1·51	0·25	0·37	0·046
Citrulline‡	27·0	12·2	8·0	23·7	16·7	15·4	11·5	16·5	5·92	0·39	0·32	0·69
Glutamic acid‡¶	87·7	91·4	110·6	84·6	49·3	78·7	60·5	54·6	10·6	<0·001	0·86	0·95
Glutamine¶**	57·6	109·2	87·7	59·8	67·0	28·6	31·3	52·6	13·7	<0·001	0·096	0·72
Glycine‡§¶	90·4	83·7	77·1	107·2	62·2	60·0	52·8	57·7	6·94	<0·001	0·84	0·001
Histidine‡	24·7	30·4	29·3	25·5	28·8	31·2	28·0	28·5	2·32	0·25	0·26	0·16
Isoleucine§¶	13·1	9·8	8·9	14·3	20·8	26·9	21·1	22·3	2·68	<0·001	0·056	<0·001
Leucine¶	9·9	8·3	6·7	9·5	34·7	43·1	33·5	36·7	3·66	<0·001	0·002	<0·001
Lysine‡	16·4	17·9	12·4	24·0	28·0	23·4	17·0	25·4	3·48	0·006	0·27	0·009
Methionine	11·5	14·1	10·2	12·1	14·3	16·4	11·5	16·1	1·62	0·019	0·002	<0·001
Ornithine	7·0	6·6	6·1	8·3	11·0	12·1	10·8	12·5	1·48	<0·001	0·54	0·007
Phenylalanine	7·9	8·0	7·5	8·4	11·2	13·4	10·6	12·0	1·10	<0·001	0·18	0·005
Serine	12·7	14·8	11·3	15·5	13·1	12·2	9·8	14·0	1·40	0·16	0·16	<0·001
Taurine	9·3	11·1	11·2	10·1	8·9	10·6	8·4	9·7	1·45	0·23	0·85	0·11
Threonine‡	25·8	25·7	16·7	30·4	26·3	27·6	20·5	28·7	3·60	0·65	0·003	<0·001
Tyrosine**	8·6	15·2	10·6	9·9	22·2	19·1	14·7	22·4	2·15	<0·001	0·017	0·001
Valine§**	66·4	48·4	44·9	77·7	82·3	97·4	79·5	84·0	9·79	<0·001	0·23	<0·001

BCAA, branched-chain amino acid.
* For details of diets and procedures, see Table 1 and text.
† SED for diet × insulin x stage of lactation. There were no significant ($P>0·05$) interactions except where indicated.
‡ Significant I × S interaction ($P<0·05$).
§ Significant D × S interaction ($P<0·05$).
¶ Significant D × I interaction ($P<0·05$).
** Significant D × I × S interaction ($P<0·05$)

an increased milk fat content. In the present work, there was no effect of BCAA on milk fat content ($P=0·91$). This is presumably because sows in Richert and coworkers study (1997) mobilised more body fat than sows in the current study (although this was not measured). Increased milk fat is a common observation as mammals move into greater energy deficits (Bauman *et al.* 1989). On the other hand, in an earlier study, Richert *et al.* (1996) found that although increasing the valine content of a relatively low-protein (143 g/kg CP) basal diet increased litter growth, there was no effect on sow live weight or fat loss. The data of Richert *et al.* (1997) showed that when sows were fed relatively low levels of dietary protein, increasing the BCAA content alone of the diet could increase milk protein and fat content as well as increase piglet growth. Our study has shown that when sows are fed diets containing a higher basal level of protein (162 g/kg CP), increasing both the dietary protein and BCAA content can increase milk protein content and piglet growth without changing the fat content or milk yield.

Despite the absence of evidence to support a direct effect of insulin on milk protein synthesis, recent systemic simultaneous infusions of insulin and dextrose have been shown to increase milk protein content and yield in dairy cows (McGuire *et al.* 1995; Griinari *et al.* 1997; Leonard & Block, 1997; Mackle *et al.* 1999, 2000*a*,*b*) and goats (Bequette *et al.* 2001). In their initial study, McGuire *et al.* (1995) observed that the circulating concentrations of amino acids, in particular BCAA, were markedly decreased, presumably because they were being preferentially oxidised or incorporated into milk proteins. Therefore, in a subsequent study, these authors supplied additional protein as an abomasal infusion of casein with and without insulin/dextrose infusions. Casein and insulin/dextrose alone increased milk protein yield by 10 % and 4 %, respectively, whereas the combination treatment increased milk protein yield by 28 % (Griinari *et al.* 1997). Although there were some increases in milk yield, the predominant response was an increase in milk protein content. Despite supplying additional protein as abomasal casein, the infusion of insulin and dextrose decreased the plasma concentrations of the BCAA valine, isoleucine and leucine by approximately 60 %. The plasma concentrations of the other essential amino acids decreased by an average of 25 % (range 13–40 %).

Since it was likely that supplying additional BCAA may have stimulated milk protein yield even further, Mackle *et al.* (2000*a*,*b*) conducted a study in which they supplied abomasal casein and supplemental BCAA. Although they were able partially to ameliorate the insulin-stimulated decrease in plasma BCAA, the improvements in milk protein yield in response to insulin and dextrose infusion were of a similar magnitude to that observed by Griinari *et al.* (1997).

In the present study, the provision of supplemental amino acids, especially BCAA, ensured that there were sufficient amino acids so that milk protein production was not limited by amino acid or BCAA supply. Insulin infusion decreased the plasma concentrations of a few key amino acids, including isoleucine (−6 %), leucine (−10 %), methionine (−11 %), threonine (−9 %) and tyrosine (−11 %), although the magnitude of these decreases was relatively small and most pronounced in sows fed the additional protein and BCAA. The apparently small effect on plasma amino acids compared with dairy cows may be related to the low insulin infusion rate employed in the present study (see below). Certainly, higher doses of insulin decrease plasma amino acids in younger growing pigs (Wray-Cahen *et al.* 1997).

Reynolds and Rook (1977) found that the infusion of both insulin (10 or 100 U/h) and glucose (10 or 20 g/h) alone increased milk protein concentrations or yield in lactating sows, but the

mechanisms appeared to be different for the two. Also, these authors did not investigate the combination of insulin and glucose, the effects of which are interdependent. In particular, the insulin infusions resulted in sustained hypoglycaemia with a resultant decreased milk lactose yield and content.

The increase in milk yield observed during simultaneous insulin and dextrose infusions in the present study, in which glycaemia was maintained, may have been due to a stimulation of milk lactose secretion, which is in turn generally accepted as being the principal osmotic determinant of milk secretion. Therefore, insulin and/or dextrose infusions may increase milk protein yield through stimulating lactose secretion. In this context, Campbell *et al.* (1990) compared the inclusion of dietary dextrose compared with fructose in the diet of lactating sows and found that the former had a greater effect on plasma insulin, both pre- and post feeding, and increased litter growth rate in multiparous but not primiparous sows. Milk lactose content was higher in the milk of sows fed a diet containing 20 % dextrose on day 18 but not 22 of lactation, whereas milk fat tended to be lower. Milk protein was not altered. It may therefore be possible to alter the insulin/glucose axis through including dextrose or sugar in the diet rather than by simultaneous infusion, as was the case in the present study.

The simultaneous infusion of dextrose and insulin caused a substantial reduction in fat mobilisation, as indicated by the reduction in plasma NEFA as occurs in ruminants and growing pigs (Dunshea *et al.* 1992, 1995; Petterson *et al.* 1993; McGuire *et al.* 1995). As a result, the milk fat content tended to be reduced in sows receiving the simultaneous infusions of insulin and dextrose. A reduction in milk fat content has also been oberved in dairy cows (McGuire *et al.* 1995; Griinari *et al.* 1997; Mackle *et al.* 1999) and goats (Bequette *et al.* 2001) receiving simultaneous infusions of insulin and dextrose. Therefore, manipulation of the insulin/glucose axis may be a method of reducing fat mobilisation and milk fat content in lactating sows. Reducing fat mobilisation would possibly aid in reducing the weaning-to-mating interval as body fatness at weaning is negatively related to weaning-to-mating interval. In addition, sow's milk has a very high fat:protein ratio, which means that much of the energy in milk is not used for growth but is converted into body fat stores.

It is interesting to note that the effects of insulin that were observed in the present study in sows in peak lactation occurred with a relatively modest (21 *v.* 31 μU/ml) proportional increment in plasma insulin that was still within the physiological range. Mid- to late-lactation dairy cows appear to have slightly higher plasma insulin concentrations than peak lactating sows (40–72 μU/ml; McGuire *et al.* 1995; Griinari *et al.* 1997; Mackle *et al.* 1999, 2000b), and most of the studies with dairy cows have involved higher insulin infusion rates than used here (11 *v.* 27 mU/kg per h), resulting in proportionately greater increases in plasma insulin (50 % *v.* 500–1000 %). A preliminary study conducted before the current experiment found that a higher infusion rate than 11 mU/kg per h required a very high dextrose infusion rate to maintain glycaemia, with a resultant dramatic reduction (substitution) in feed intake. For example, during this preliminary study, insulin and dextrose rates infusion rates of 27 mU/kg per h and 2 kg/d were associated with a 3 kg/d decrease in feed intake.

Leury *et al.* (2003) conducted a study in early-lactation dairy cows and found that they had plasma insulin levels (19 μU/ml) similar to those observed here in early-lactation sows. These authors also had reduced feed intakes in their cows when infusions went beyond 2 d (BJ Leury, personal communication), and it is tempting to speculate that animals that are in early lactation and exhibit low endogenous insulin concentrations may be particularly sensitive to the effects of insulin on feed intake. Alternatively, it may be that pigs are much more sensitive to insulin than ruminants (Pethick & Dunshea, 1996; Dunshea & D'Souza, 2003). Dunshea & D'Souza (2003) collated data from a number of *in vitro* studies and concluded that adipose tissue from pigs was much more sensitive to insulin than adipose tissue from ruminants. Pethick and Dunshea (1996) reached a similar conclusion based on whole-body glucose responses to exogenous insulin infusion.

In conclusion, these data demonstrate that increasing the dietary protein/BCAA content of the sow diet can increase milk protein secretion but not milk yield. A combined infusion of insulin and dextrose can increase milk, milk lactose and milk protein yields but not milk protein content. These effects are additive and translate to increased piglet growth. Further research is necessary to separate the dextrose and insulin effects and to determine the optimal way to modulate the insulin/dextrose/BCAA axis to increase milk protein yield and piglet growth on the farm.

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References

Atwood CS & Hartmann PE (1992) Collection of fore and hind milk from the sow and changes in milk composition during suckling. *J Dairy Res* **59**, 287–298.

Auldist DE, Stevenson FL, Kerr MG, Eason PJ & King RH (1997) Lysine requirements of pigs from 2 to 7 kg live weight. *Anim Sci* **65**, 501–507.

Bauman DE, Dunshea FR, Boisclair YR, McGuire MA, Harris DM & Houseknecht KL (1989) Regulation of nutrient partitioning: homeostasis, homeorhesis and exogenous somatotropin. Proceedings of the Seventh International Conference on Production Disease in Farm Animals, FA Kallfelz (ed.). Ithaca, NY: Cornell Univ Press, pp. 306–323.

Bequette BJ, Kyle CE, Crompton LA, Buchan V & Hanigan MD (2001) Insulin regulates milk production and mammary gland and hind-leg amino acid fluxes and blood flow in lactating goats. *J Dairy Sci* **84**, 241–255.

Campbell WJ, Brendemuhl JH & Bazer FW (1990) Effect of fructose consumption during lactation on sow and litter performance and sow plasma constituents. *J Anim Sci* **68**, 1378–1388.

Dunshea FR (2003) Metabolic and endocrine changes around weaning. In *Weaning the Pig*, pp. 61–80 [JR Pluske, J Le Dividich and MVA Verstegen, editors]. Wageningen, The Netherlands: University of Wageningen Press.

Dunshea FR & D'Souza DN (2003) Factors influencing fat deposition and metabolism in the pig. In *Manipulating Pig Production IX*, pp. 127–150 [J Paterson, editor]. Werribee, Australia: Australasian Pig Science Association.

Dunshea FR & King RH (1995) Responses to homeostatic signals in ractopamine-treated pigs. *Br J Nutr* **73**, 809–818.

Dunshea FR, Boisclair YR, Bauman DE & Bell AW (1995) Effects of bovine somatotropin and insulin on whole-body and hindlimb glucose metabolism in the growing steer. *J Anim Sci* **73**, 2263–2271.

Dunshea FR, Harris DM, Bauman DE, Boyd RD & Bell AW (1992) Effect of somatotropin on non-esterified fatty acid and glycerol metabolism in growing pigs. *J Anim Sci* **70**, 132–140.

Glencross BD, Mullan BP, Tuckey RC & Hartmann PE (1997) A simplification of the deuterium oxide dilution technique using FT-IR analysis of plasma, for estimating piglet milk intake. *Aust J Agric Res* **48**, 1099–1104.

Griinari JM, McGuire MA, Dwyer DA, Bauman DE, Barbano DM & House WA (1997) The role of insulin in the regulation of milk protein synthesis in dairy cows. *J Dairy Sci* **80**, 2361–2371.

Hove K (1978) Maintenance of lactose secretion during acute insulin deficiency in lactating goats. *Acta Physiolog Scandin* **103**, 173–179.

King RH, Rayner CJ & Kerr M (1993*a*) A note on the amino acid composition of sow's milk. *Anim Product* **57**, 500–502.

King RH, Toner MS, Dove H, Atwood CS & Brown WG (1993*b*) The response of first-litter sows to dietary protein level during lactation. *J Anim Sci* **71**, 2457–2463.

Laarveld B, Christenson DA & Brockman RP (1981) The effect of insulin on net metabolism of glucose and amino acids by the bovine mammary gland. *Endocrinology* **108**, 2217–2221.

Laarveld B, Chaplin RK & Brockman RP (1985) Effects of insulin on metabolism of acetate, beta-hydroxy butyrate and triglycerides by the bovine mammary gland. *Comp Physiol Biochem* **82B**, 265–267.

Leonard M & Block E (1997) Effects on nutrient and hormonal profile of long-term infusions of glucose or insulin plus glucose in cows treated with recombinant bovine somatotropin before peak milk yield. *J Dairy Sci* **80**, 127–143.

Leury BJ, Baumgard LH, Block SS, Segoale N, Ehrhardt RA, Rhoads RP, Bauman DE, Bell AW & Boisclair YR (2003) Effect of insulin and growth hormone on plasma leptin in periparturient dairy cows. *Am J Physiol* **285**, R1107–R1115.

McGuire MA, Griinari JM, Dwyer DA & Bauman DE (1995) Role of insulin in the regulation of mammary synthesis of fat and protein. *J Dairy Sci* **78**, 816–824.

Mackle TR, Dwyer DA & Bauman DE (2000*a*) Intramammary infusion of insulin or long R3 insulin-like growth factor-I did not increase milk protein yield in dairy cows. *J Dairy Sci* **83**, 1740–1749.

Mackle TR, Dwyer DA, Ingvartsen KL, Chouinard PY, Lynch JM, Barbano DM & Bauman DE (1999) Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. *J Dairy Sci* **82**, 1512–1524.

Mackle TR, Dwyer DA, Ingvartsen KL, Chouinard PY, Ross DA & Bauman DE (2000*b*) Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. *J Dairy Sci* **83**, 93–105.

Mellor DJ & Cockburn F (1986) A comparison of energy metabolism in the new-born infant, piglet and lamb. *Q J Exp Physiol* **71**, 361–379.

Ostrowska E, Cross RF, Muralitharan M, Bauman DE & Dunshea FR (2002) Effects of dietary fat and conjugated linoleic acid on plasma metabolite concentrations and metabolic responses to homeostatic signals in pigs. *Br J Nutr* **88**, 625–634.

Pethick DW & Dunshea FR (1996) The partitioning of fat in farm animals. *Proc Nutr Soc Aust* **20**, 3–13.

Petterson JA, Dunshea FR, Ehrhardt RA & Bell AW (1993) Pregnancy and undernutrition alter glucose metabolic responses to insulin in sheep. *J Nutr* **123**, 1286–1295.

Prawirodigdo S, King RH, Dunkin AC & Dove H (1990) Evaluation of techniques for estimating milk production by sows. 1. Deuterium oxide dilution method for estimating milk intake by piglets. *Asian-Austr J Anim Sci* **3**, 135–141.

Rayner C (1985) Protein hydrolysis of amino feeds for amino acid content. *J Agric Food Chem* **33**, 725–731.

Reeds PJ & Mersmann HJ (1991) Protein and energy requirements of animals treated with beta-adrenergic agonists: a discussion. *J Anim Sci* **69**, 1532–1550.

Reynolds L & Rook JAF (1977) Intravenous infusion of glucose and insulin in relation to milk secretion in the sow. *Br J Nutr* **37**, 45–53.

Richert BT, Goodband RD, Tokach MD & Nelssen JL (1997) Increasing valine, isoleucine, and total branched-chain amino acids for lactating sows. *J Anim Sci* **75**, 2117–2128.

Richert BT, Tokach MD, Goodband RD, Nelssen JL, Pettigrew JE, Walker RD & Johnston LJ (1996) Valine requirement of the high-producing lactating sow. *J Anim Sci* **74**, 1307–1313.

Rutherfurd SM & Moughan PJ (1998) The digestible amino acid composition of several milk proteins: application of a new bioassay. *J Dairy Sci* **81**, 909–917.

Tokach MD, Pettigrew JE, Crooker BA, Dial GD & Sower AF (1992) Quantitative influence of lysine and energy intake on yield of milk components in the primiparous sow. *J Anim Sci* **70**, 1864–1872.

Toner MS, King RH, Dunshea FR, Dove H & Atwood CS (1996) The effect of exogenous somatotropin on lactation performance of first-litter sows. *J Anim Sci* **74**, 167–172.

Williams IH (1976) *Nutrition of the young pig in relation to body composition*. Thesis submitted for the degree of Doctor of Philosophy, University of Melbourne, Australia.

Wray-Cahen D, Beckett PR, Nguyen HV & Davis TA (1997) Insulin-stimulated amino acid utilization during glucose and amino acid clamps decreases with development. *Am J Physiol (Endocrinol Metab)* **273**, E305–E314.