IN VITRO BRANCHED CHAIN AMINO ACID OXIDATION BY PORCINE MAMMARY TISSUE

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Summary

Mammary secretory tissue from six (three each of parity 1 and 2) lactating sows (d 10 to 17 of lactation) was obtained via biopsy for in vitro incubation to determine CO_2 production from individual branched chain amino acids. Carbon dioxide production levels as percentages of the ^{14}C-labeled amino acid metabolized by the mammary tissue were 2.57, 1.86, and 4.07% for isoleucine, leucine, and valine, respectively ($P < .03$). These results indicate that, in the lactating sow mammary gland, valine has the greatest oxidation rate of the branched chain amino acids.

(Key Words: Mammary Gland, Sows, Isoleucine, Leucine, Valine.)

Introduction

The lactating cow and goat have received more emphasis in determining nutrient utilization by the mammary gland than the lactating sow. This is because of the difficulty of cannulating the sow's mammary glands, which are supported metabolically through many feeder arteries and veins. Because of this difficulty, mammary biopsy and in vitro culture of mammary tissue offer potential to study utilization of nutrients. Research reported in the 1994 Swine Day Report of Progress (p 10 and 15) demonstrated that the dietary valine requirement of the high-producing lactating sow is higher than NRC (1988) and ARC (1981) estimates. Additionally, the known differences between species in milk profile, with swine having greater DM, lipid, and protein than dairy cattle, indicates the potential for different metabolic use of the branched chain amino acids. Therefore, the objective of this experiment was to determine the in vitro CO_2 production from ^{14}C labeled L-valine, L-isoleucine, and L-leucine by sow mammary tissue.

Procedures

Six sows (half parity 1 and half parity 2) of maternal line genetics (PIC Line C15) were used. The first and second productive mammary glands on the right side of the sow were biopsied to collect mammary tissue. Sows were between d 10 and 17 of lactation at time of the biopsy. Sows were allowed ad libitum access to an experimental diet high in all three branched chain amino acids. The diet was formulated to contain .90% lysine, .85% isoleucine, 1.35% leucine, and 1.07% valine.

The incubation medium used in this experiment was a RPMI-1640 select amine® kit (Life Technologies, Grand Island, NY). The medium was complete, with no antibiotics, fetal bovine serum, or supplemental hormones because of the short duration of the incubation. All nutrients were mixed in the medium except isoleucine, leucine, and valine. After purification, the medium was aseptically divided into four equal vials with a sterile syringe, needle, and .2 micron filter. One vial was deficient in each of the three amino acids and the fourth deficient in all three (for transportation of tissue biopsies). The individual branched chain amino acids were added to their respective vials using the

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same aseptic technique as described above. All radioisotopes used were at an activity of 100 μCi/mL. However, each isotope had a slightly different molar concentration: isoleucine, 240 mCi/mmol; leucine, 324.9 mCi/mmol; and valine, 225 mCi/mmol. The initial medium pH was 7.4.

Approximately equal weights of tissue from each gland (100 to 150 mg) were used. The tissue slices (approximately 2 to 3 mm^3 and 50 mg) were weighed and placed in a 50 mL flask with 5 mL of tissue medium. Five μL of ^{14}C-U-valine, isoleucine, or leucine (100 μCi/ml) was added to the medium. The flask then was purged with 95:5 (oxygen: CO_2) for 1 min, sealed with a rubber stopper, and placed in a water bath. The rubber stopper contained a suspension center well with 2^2 cm piece of filter paper inside it. The tissue samples were incubated in a 37°C shaker water bath for 1 h. Then .3 mL of 2 M KOH was injected through the rubber stopper into the center well to capture CO_2 released when the incubation period was terminated. The tissue incubation then was terminated by the injection of 1 mL of 1 N H_2SO_4 into the medium. The tissue then was incubated again for another 1 h period for CO_2 collection.

After CO_2 collection, the flasks were removed from the water bath, and the center wells were placed in a 6 mL liquid scintillation vial. Tissue was removed from the vial and rinsed with 2 mL of distilled deionized water, with the rinsing added to the incubation medium. The rinsed tissue then was placed in 5 mL of distilled deionized water and homogenized for 4 min. using a Tekmar Tissuemizer®. Fifty μL of homogenized tissue and 40 μL of medium + washings were pipetted into liquid scintillation vials, to which 5 mL of scintillation fluid was added before they were counted on the next day. Sample degradations were counted for 4 min. on a Tri-carb 4000® liquid scintillation counter. Quench curves were performed for all three types of samples. The external standardization method using the spectral index of the sample was used to generate the counting efficiency of the samples.

Results and Discussion

The ^{14}C concentration in CO_2 was not different between branched chain amino acids ($P < .33$). When CO_2 production was expressed as a percentage of label incorporated into tissue, valine had the highest CO_2 production (4.07%) followed by isoleucine (2.57%) and leucine (1.57%). These values are similar to those reported in the literature for total ^{14}C recovered as CO_2. However, contrary to our results, in lactating bovine mammary tissue, leucine has greater CO_2 production followed by isoleucine and then valine. This suggests that species differences occur in the metabolism of the branched chain amino acids.

Valine had the lowest incorporation rate ($P < .001$) of the branched chain amino acids into the mammary tissue when measured as quantity of ^{14}C in the tissue homogenate. However, when comparing the branched chain amino acids on a molar concentration basis of radioactive amino acid, no differences occurred ($P < .16$). However, isoleucine had a numerically higher incorporation rate than valine or leucine when considered on the molar basis.

Production of labeled CO_2/mg/h was similar between parities. However, parity 1 sows had greater labeled CO_2 production as a percent of the branched chain amino acid metabolized (3.4 vs 2.3%) than parity 2 sows. Parity 2 sows had 46% greater uptake rates of the branched chain amino acids than parity 1 sows, accounting for most of the difference in CO_2 as a percentage of branched chain amino acids extracted. The increased uptake rates by parity 2 sows is related to the greater milk synthesis rates observed with increasing parity, as the sow reaches her maximum productivity. Also, the higher CO_2 production by the parity 1 sows may relate to greater activation of catabolism enzymes because of their smaller BW and their need to use more of the branched chain amino acids for energy.

In conclusion, in vitro CO_2 production rates in sow mammary tissue were greatest for valine and least for leucine. Isoleucine

appears to have the greatest uptake rate by mammary tissue of the branched chain amino acids. Of the previously reported 30 to 80% excess uptake of the branched chain amino acids by the mammary gland above the requirement for milk protein synthesis, only a small fraction appears to be utilized strictly as an energy source. This suggests that the branched chain amino acids play a large part as carbon and nitrogen donors for synthesis of nonessential amino acids, lactose, and(or) lipid.

Table 1. In Vitro CO_2 Production from ^{14}C-L-isoleucine, Leucine, and Valine by Sow Mammary Tissue

	Amino acid				
Item	Isoleucine	Leucine	Valine	SE	P <
Added amino acid, nmol	2.08	1.5	2.2	—	—
Tissue wt, mg	281.5	278.1	295.6	18.2	.77
Radio label recovery, %					
Residual media	59.89	60.73	76.24	1.85	.001
Tissue homogenate	19.45	21.44	13.15	1.31	.001
CO_2	.50	.39	.55	.06	.33
Total	79.84	82.52	89.94	1.57	.001
CO_2 as a percentage of uptake	2.57	1.86	4.07	.55	.03
Tissue uptake rate, pmol/mg/h	1.11	.91	.89	.004	.16
CO_2 production rate, pmol/mg/hr	.0259	.0114	.0315	.09	.02

[a]Means represent values from six sows for a 1 h incubation at 37°C conducted in duplicate.

Table 1. Salmonella Serotype and Source

Salmonella species	Source
S. choleraesuis	1 finisher pig, 1 water bowl
S. agona	8 fecal swabs (finishers), 5 environment
S. derby	6 environment, 1 finisher pig
S. drypool	2 environment, 1 finisher pig
S. anatum	1 fecal swab (nursery pig)
S. brandenburg	1 fecal swab (sow)
S. heidelberg	1 fecal swab (sow)

Table 2. Samples for *Salmonella* Isolation and the Number of Isolates

Sample	# Cultured	# Isolates
Milk	9	0
Fecal swabs	88	12
Pigs at necropsy	6	3
Environment	24	13
Rat tissues	9	0
Total	136	28

Discussion

These results demonstrate that SEW can drastically reduce *salmonella* shedding, control clinical salmonellosis, and possibly eliminate *salmonella* infection. *Salmonella* species were never isolated from any of the SEW pigs, and none of the pigs displayed any clinical evidence of salmonellosis. On the other hand, *S. agona* was isolated from fecal swabs from one of 15 on-farm, age-matched pigs when moved from the nursery and from seven of 13 age-matched pigs 40 d later. In addition, *S. drypool* and *S. derby* were grown from the two necropsied finisher pigs, and four species of *Salmonella* were isolated from their environment. Fourteen of 21 (67%) environmental samples were *Salmonella* positive. The positive samples consisted of water from mud holes and water bowls; *Salmonella* was not isolated from water taken directly from the hydrants. It appears that several of the *Salmonella* species were being cycled between the pigs and the environment. It is interesting that *S. choleraesuis*, except for the vaccine strain, was not isolated from any pig or their environment after the initiation of vaccination at weaning. The farm's owner felt that the vaccine was responsible for cessation of clinical signs of salmonellosis. Whether the vaccine or some other unknown factor was responsible remains unknown. Among *Salmonella* species, *choleraesuis* is by far the most common cause of death and clinical disease. The other species that were isolated from the farm, with the possible exception of *S. agona*, probably did not cause any prob-

lems to the pigs, but they represent potential sources of contamination of pork at slaughter. Thus, eliminating them is important from a food safety standpoint.

When we sampled the finisher pigs, they had a serious problem with diarrhea and poor growth. The primary cause of this was porcine proliferative enteritis, although *S. agona* may have contributed to the problem. When we initiated the project, we were interested only in elimination of *S. choleraesuis*, which appeared to be the farm's primary problem. Proliferative enteritis had not been diagnosed for some time. Therefore, samples were not saved for identification of its cause, Ileal Symbiont Intracellularis, so that we do not know if the organism was present. In any event, the SEW pigs displayed no clinical signs of proliferative enteritis, and no gross or microscopic lesions of proliferative enteritis were seen in the six pigs that were euthanized, indicating that the disease had been effectively controlled, if not eliminated.

The presence of an antibody response in infected pigs usually is recognized as a much more sensitive method of detecting *S. choleraesuis* than culture. The majority of pigs were serologically positive for *S. choleraesuis* when weaned, but all were negative by the third bleeding at 83 d after weaning. This indicates antibodies were transferred from the sows' colostrum to the piglets and that they had not been infected. Because antibody titers of two pigs were in the high suspicious range 26 d later, we cannot be sure that *S. choleraesuis* was eliminated totally. Since performing the serologic analyses, Dr. Kramer has found that his test cross reacts with other *Salmonella* species and possibly other non-salmonella bacteria. He is modifying the test so that it will be *S. choleraesuis*-specific and, when he has the test perfected, he will redo our sera to determine if the response in the two pigs was indeed to *S. choleraesuis*. Because *S. choleraesuis* occasionally can infect pigs in utero, it does not seem likely that SEW would work all the time. If the pigs are infected at birth or if the sow is actively shedding the organism, early weaning probably would not eliminate the organism. Two isolates, *S. brandenburg* and *S. heidelberg*, were grown from 44 sow fecal cultures, and both were present in very low numbers, because prolonged incubation in enrichment broth was required for each isolate. Neither isolate was cultured from the environment or the growing pigs. Recent work at the National Animal Disease Center indicates that SEW will work only if the sows are not actively shedding *Salmonella* species during the nursing period. If SEW is to work, pigs should be weaned into facilities that can be totally emptied and thoroughly cleaned and disinfected between each group. Then salmonellosis does occur in a group of pigs, environmental carryover between groups will be prevented, and infected pigs from an earlier group will not serve as a source of infection for new pigs.