Effects of Abomasal or Intravenous Administration of Arginine on Milk Production, Milk Composition, and Concentrations of Somatotropin and Insulin in Plasma of Dairy Cows

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ABSTRACT
Holstein cows just past peak lactation were used in a 3 x 3 Latin square design to determine the effects of arginine infusion on concentrations of somatotropin and insulin in plasma, milk production, and milk composition. Treatments were: 1) control; 2) arginine injection into jugular vein, and 3) arginine infusion into abomasum. Concentrations of arginine and ornithine in plasma were increased by injection of arginine into the jugular vein compared with the control. The concentration of ornithine in plasma was increased shortly after injection of arginine into the jugular vein, and both arginine and ornithine concentrations in plasma decreased rapidly. Abomasal infusion of arginine significantly increased concentrations of arginine, ornithine, and urea in plasma compared with concentrations in the control treatment. Injection of arginine into the jugular vein increased concentrations of somatotropin and insulin in plasma, but the increase did not persist for more than 30 min. A secondary peak in plasma somatotropin concentration occurred approximately 1 h after the initial peak. Arginine infusion into the abomasum did not alter plasma concentrations of either somatotropin or insulin. Dry matter intake, milk production, and milk composition were not affected by treatments. Lack of changes in milk production and milk composition suggest that acute increases in somatotropin with concomitant increases in insulin are not sufficient to stimulate synthesis of milk and milk components by cows during established lactation.

INTRODUCTION
Changes in plasma concentrations of galactopoietic hormones have been suggested as a mechanism for the increase in milk production that occurs when lactating cows are given abomasal infusions of protein (6). Intravenous injection of arginine increases plasma concentrations of somatotropin (bST), insulin, prolactin, and placental lactogen in plasma of ruminants (5, 8, 17, 18, 19, 23, 24). In those trials, each intravenous injection of arginine was for a short time, usually about 5 min, and produced a brief increase in plasma hormone concentrations. Daily injections of .1 g L-arginine/kg body weight into the jugular vein of 8 cows during a 4 to 5-min period for about 7 d just prior to parturition caused plasma concentrations of bST, prolactin, and insulin to increase and these hormonal responses did not become refractory (5). Although arginine was not injected postpartum, average daily milk production was 10% greater during the first 22 wk of lactation for four cows injected with arginine prepartum than for five cows injected with saline prepartum. Chew et al. (5) suggested that the increase in milk production was caused by the increased plasma concentrations of lactogenic hormones prepartum.

Few experiments have been conducted to investigate the effect of arginine infusion into the abomasum or small intestine on plasma hormone concentrations in ruminants, and these experiments were of short duration. Milk production and concentrations of bST in plasma were not altered when 25 g of arginine were infused into the abomasum of goats; however, arginine was infused for only 1 h/d...
for a total of 12 d (13). Oldham et al. (26) also
did not detect changes in plasma concentrations
of bST during a 24-h infusion of 23.8 g of
arginine into the abomasum of lactating goats.
The effects of long-term infusions of arginine
into the abomasum or blood of lactating cows
have not been reported. The objectives of this
experiment were to determine effects of
continuous abomasal infusions and pulse
intravenous injections of arginine on plasma
concentrations of bST and insulin and on yield
and composition of milk.

MATERIALS AND METHODS

Animals and Treatments

Three ruminally cannulated Holstein cows
73 to 87 d postpartum and weighing 555, 520,
and 557 kg were used in a 3 × 3 Latin square
design to evaluate abomasal and intravenous
administration of arginine on concentrations
of hormones in plasma and its effects on milk
yield and composition. Catheters were inserted
into the jugular vein 1 d prior to the start of
each period using polyvinyl tubing. An infusion
tube was placed into the abomasum (31)
Adv. the start of the experiment and its location
was verified at the start and end of each period.
Treatments consisted of: 1) saline infusion into
the abomasum and injection into the jugular
vein (control); 2) arginine injection into the
jugular vein and saline infusion into the abo-
masum; and 3) saline injection into the jugular
vein and arginine infusion into the abomasum.
Arginine was injected intravenously to provide
0.1 g L-arginine-HCl/kg body weight (35%
wt/vol) twice daily at 0730 and 1930 h. This
resulted in 111, 104, or 112 g/d of arginine
injected into each of the three cows. Arginine
was injected through the jugular catheter with a
syringe over approximately 5 min. Arginine (50
mg/ml) was infused continuously into the
abomasum and supplied an average of 178 g/d
to each cow. Saline infusions into the ab-
omasum and injections into the jugular vein
were of similar volume and duration. All
infusion solutions were adjusted to pH 7.4.
Cows were assigned randomly to treatments.
Each of the three treatment periods lasted 6 d
with 5 d between infusion periods.

Diets

Cows were fed ad libitum a total mixed
ration containing 55% concentrate, 26% alfalfa-
grass haylage, and 19% corn silage. The con-
centrate consisted of 79.1% shelled corn, 16.6%
soybean meal, .5% mineral and vitamins, 1.0%
dicalcium phosphate, .1% magnesium oxide,
1.4% limestone, .5% sodium sulfate, and .8%
sodium chloride. The total mixed diet con-
tained 15.3% CP and 17.8% ADF. The diet was
offered daily at 0600 and 1800 h. Orts were
weighed at 1630 h. Cows were milked daily at
0500 and 1700 h. Feeds and orts were sampled
on the 3rd d of each period. Samples were dried
at 55°C in a forced air oven and ground in a
Wiley mill to pass a 2-mm screen. Samples were
analyzed for CP (2) and ADF (11). Milk sam-
ple were taken at each milking, preserved with
potassium dichromate, and stored at 4°C until
analysis. Samples were analyzed for fat, protein
(Multispec A, infrared milk analyzer), and
solids-not-fat (12).

Plasma Samples and Assays

On the last day of each period, 15-ml blood
samples were obtained in tubes containing 300
IU of sodium heparin in 100 μl of saline at
15-min intervals from 1845 to 2130 h. Samples
were taken before 1930 h to determine whether
there were chronic effects of the morning
injection on plasma amino acids or hormones in
cows getting intravenous injections of arginine.
Additional blood samples were taken at 30-min
intervals from 2200 to 2300 h. Blood samples
were centrifuged within 10 min after with-
drawal to obtain plasma. A portion of the
plasma samples obtained at 1845, 1945, 2130,
and 2300 h were deproteinized immediately by
mixing 3 ml of plasma with .3 ml of 50% sul-
fosalicylic acid. Samples were mixed, frozen
overnight, thawed, and centrifuged at 10,000 x
× g for 15 min. Supernatant was transferred to a
vial and frozen at −70°C until analyzed for
amino acids. Amino acid analyses were by
automated amino acid analyzer (Beckman
Model 6300, Fullerton, CA). Norleucine was
added to each sample as an internal standard
prior to applying the sample to the column.
The remaining plasma was stored at −20°C
until analyzed for bST and insulin concen-
trations. Bovine somatotropin was analyzed by
double antibody radioimmunoassay. Antibody
(GH-2) and bST (USDA-bGH-I-1) used for
iodination and standards in the assay were
supplied by National Institute of Arthritis,
Diabetes and Digestive and Kidney Diseases
Iodinated bST was prepared using iodogen (29). Antibody was used at a final dilution of 1:20,000. Primary incubation was for 48 h at room temperature and secondary incubation was for 24 h at 4°C after addition of second antibody (goat antirabbit immunoglobulin, 1:15, Miles Laboratory Elkhart, IN). The assay was validated by measuring recovery of added bST and determining parallelism. Assay sensitivity was 1.5 ng/ml and intraassay and interassay coefficients of variation averaged 8.3 and 13.2%, respectively. First antibody was tested for crossreactivity and specificity by NIADDK. Insulin was assayed by solid phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Bovine insulin standards (Sigma Chemical Co., St. Louis, MO) were substituted for human insulin standards. The assay was validated by measuring recovery of added insulin and determining parallelism. Intraassay and interassay coefficients of variation were 3.4 and 10.6%, respectively.

Statistical Analysis

Production data were analyzed statistically as a Latin square design (one observation per cow x period combination) (28). Blood data were analyzed using the Latin square model to determine effects at individual sampling times and repeated measures analysis was used to determine overall effects during the sampling period (28). Treatment comparisons were by least significant difference (LSD following significant F-test) (32).

RESULTS AND DISCUSSION

Plasma Amino Acids

The peak concentration of arginine in plasma of lactating dairy cows was elevated 13-fold (P<.05) by intravenous injection of arginine compared with injection of saline (Figure 1). Arginine was cleared rapidly from plasma and the concentration declined to values approximately equal to that of control animals by 3.5 h after arginine injection. Arginine was probably catabolized to ornithine because the rise and decline in plasma concentrations of arginine paralleled those of the ornithine concentration. Continuous infusion of arginine into the abomasum also increased plasma concentrations of arginine and ornithine compared with the infusion of saline, but the increase was significant only for ornithine (Figure 1). Urea concentrations in plasma were highest in cows that were abomasally infused with arginine, intermediate for cows injected intravenously with arginine, and lowest for control cows (Figure 1). Elevated concentrations of arginine, ornithine, and urea in plasma of cows that received continuous infusions of arginine into the abomasum suggest that large amounts of infused arginine were absorbed from the small intestine. Mean concentrations of other amino acids in plasma were
not different among treatments (Table 1). Treatment comparisons for concentrations of amino acids where cows were injected with arginine in the jugular vein were not different when the samples taken before the intravenous injection of arginine were not included. Although not significantly different among treatments, proline concentrations in plasma (Table 1) were elevated consistently after arginine was injected, suggesting that arginine also was catabolized by the glutamate semialdehyde pathway. Apparent differences in the preinjected values for amino acids from cows given intravenous administration of arginine were the result of carryover from the intravenous arginine injection 11 h, 15 min earlier.

### Plasma Hormones

The pattern of bST concentration in plasma was altered by the intravenous injection of arginine (Figure 2). Plasma concentrations of bST were increased \((P<.05)\) 45 min after intravenous injection of arginine to approximately five times the concentration of bST in plasma of control cows. One hour after intravenous injection of arginine, bST returned to near baseline concentrations. This decline in plasma bST concentration was followed by a delayed increase \((P<.05)\) in concentration of bST that persisted for at least 1 h. Mean concentrations of bST during the sampling period were 2.87, 3.74, and 5.87 ng/ml for cows given control, abomasal, and jugular infusions,

<table>
<thead>
<tr>
<th>Amino acid, µg/ml</th>
<th>Control</th>
<th>Abomasum²</th>
<th>Jugular vein³</th>
<th>SEM</th>
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<td>Aspartic acid</td>
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<td>.27</td>
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<td>Glycine</td>
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<td>17.03</td>
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<td>.15</td>
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<td>Leucine</td>
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<td>6.90</td>
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<td>9.43b</td>
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<td>Lysine</td>
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<td>3-Methylhistidine</td>
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<td>.42</td>
<td>.58</td>
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<td>Carnosine</td>
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<td>3.78</td>
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<td>Urea, mg/dl</td>
<td>13.8¹</td>
<td>21.9b</td>
<td>18.1ab</td>
<td>1.22</td>
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</table>

¹,²Values within the same row with different superscripts differ \((P<.05)\).

³Mean of samples taken at 1845, 1945, 2130 and 2300 h. Each value is the mean of observations from three cows.

‡Indicates 178 g L-arginine-HCl/cow per d infused continuously into abomasum.

³Indicates .1 g L-arginine-HCl/kg body weight was administered into jugular vein twice daily at 0730 and 1930 h.
respectively. These concentrations were not different (P>.10). Plasma concentrations of insulin were increased (P<.05) immediately after the intravenous injection of arginine and remained elevated for at least 30 min (Figure 3). Mean concentrations of insulin in plasma during the sampling period for cows given intravenous injections of arginine were significantly higher than for control cows (2.77 vs. 1.20 ng/ml). Mean concentration of insulin from cows that received abomasally infused arginine (2.04 ng/ml) was not different (P>.10) from other treatments. Thus, alterations in hormones observed in this study were acute in response to the intravenous injections of arginine.

The rapid increase in plasma bST and insulin concentrations has been observed by other investigators when arginine was administered intravenously into ruminants (5, 8, 17, 18, 19, 23, 24). The second bST peak in plasma obtained in our trial when arginine was administered intravenously (Figure 2) was similar to the pattern of ovine somatotropin (oST) in plasma obtained by Handwerger et al. (14) when arginine was injected into the jugular vein of sheep. Factors responsible for this delayed peak in plasma somatotropin concentration have not been investigated. Arginine injection into sheep produced a delayed increase in plasma concentrations of placental lactogen (14). The delayed increase in plasma concentrations of oST and placental lactogen suggests that a metabolite of arginine may cause the increase in plasma concentrations of these two hormones. Intravenous injection of ornithine, a metabolite of arginine, increases plasma concentrations of oST and placental lactogen in sheep (15) and might be involved in the delayed arginine-induced increase in oST and placental lactogen. Approximately a 1 h delay was observed before oST concentrations peaked in plasma when ornithine was intravenously infused in sheep. In the present experiment,
plasma concentrations of ornithine were increased 15 min after intravenous injection of arginine (Figure 1). Ornithine is produced by cleavage of urea from arginine by arginase. Thus, the increased concentration of ornithine in the plasma may be responsible for the delayed peak of bST in plasma after intravenous injection of arginine.

Although the pattern of bST in plasma of cows in our experiment was changed, the effects of pattern of bST concentrations in plasma on biological responses are not well understood in the bovine species. Sexually dimorphic patterns of bST concentrations in plasma of rats may contribute to differences in weight gain of male and female rats (22). However, sexual differences in bST concentrations in the bovine species have not been investigated thoroughly. Administration of exogenous bST to lactating dairy cows increased milk production (4) and the pattern of bST administration (single subcutaneous injection, continuous subcutaneous infusion, or six intravenous injections daily) did not affect the positive response (10).

**Milk Production**

Alteration of plasma hormone concentrations (Figures 2 and 3) by intravenous arginine injection did not alter milk production or milk composition (Table 2). The lack of a production response may be attributed to several factors. First, the increased plasma concentration of bST may not have been maintained long enough or at a high enough concentration to increase milk production. Our findings are similar to results obtained when a fragment of somatotropin-releasing factor (SRF) was intravenously injected into lactating dairy cows (25). The SRF fragment increased plasma concentrations of bST but did not induce increases in bST of sufficient magnitude to stimulate milk production when compared with results from trials in which exogenous bST was administered. Second, although bST is known to be a potent galactopoietic hormone, insulin has been demonstrated to have a negative influence on lactation (21). Therefore, any beneficial effect resulting from the increased plasma bST concentration (Figure 2) may have been counteracted by an increased concentration of insulin in plasma (Figure 3). Third, even though Chew et al. (5) infused arginine intravenously into cows during the prepartum period and increased (P<.10) milk production during the following lactation, the response may have been mediated by factors other than bST. Intravenous injection of

### Table 2. Effects of abomasal and intravenous infusions of arginine on feed intake, milk production and composition, and efficiency of feed utilization.¹,²

<table>
<thead>
<tr>
<th>Site of arginine infusion</th>
<th>Control</th>
<th>Abomasum³</th>
<th>Jugular vein⁴</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake, kg/d</td>
<td>20.5</td>
<td>20.2</td>
<td>21.0</td>
<td>.5</td>
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<tr>
<td>Milk, kg/d</td>
<td>30.7</td>
<td>33.4</td>
<td>31.9</td>
<td>1.5</td>
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<tr>
<td>Fat, kg/d</td>
<td>.88</td>
<td>1.02</td>
<td>.98</td>
<td>.09</td>
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<tr>
<td>Protein, kg/d</td>
<td>.94</td>
<td>.91</td>
<td>.93</td>
<td>.02</td>
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<td>Solids-not-fat, kg/d</td>
<td>2.56</td>
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<td>Fat, %</td>
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<td>Protein, %</td>
<td>3.08</td>
<td>2.81</td>
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<td>Solids-not-fat, %</td>
<td>8.36</td>
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<td>.36</td>
</tr>
<tr>
<td>4% FCM, kg/d</td>
<td>25.5</td>
<td>28.7</td>
<td>27.5</td>
<td>1.3</td>
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<tr>
<td>Feed efficiency, FCM/dry matter intake</td>
<td>1.24</td>
<td>1.40</td>
<td>1.31</td>
<td>.03</td>
</tr>
</tbody>
</table>

¹ Each value is the mean of observations from three cows.
² Treatment differences were not significant (P>.10).
³ Indicates 178 g L-arginine-HCl/cow per d infused continuously into abomasum.
⁴ Indicates .1 g L-arginine-HCl/kg body weight was administered into jugular vein twice daily at 0730 and 1930 h.

arginine into these cows increased plasma concentrations of bST, insulin, and prolactin (5). The role of endogenous prolactin in galactopoiesis is not well understood. In ruminants, a reduction of prolactin concentration in plasma during lactation did not decrease milk production (20, 30) and exogenous administration of prolactin during lactation did not increase milk production (27). However, a prepartum surge in prolactin is required for successful lactogenesis in ruminants (1), and the concentration of prolactin during the prepartum period is positively and significantly correlated with postpartum milk production (9).

Clark (6) suggested that changes in plasma concentrations of amino acids that affect galactopoietic hormones may be responsible for the increase in milk production when protein is infused postruminally. In our experiment, continuous infusion of arginine into the abomasum of dairy cows doubled the concentration of arginine and ornithine (Figure 1, Table 1) in plasma of infused cows compared with that of control animals, but mean concentrations of bST and insulin over a 4-h period, milk production, and milk composition (Table 2) were not altered (P > .10). This is consistent with experiments in which arginine was continuously infused into the abomasum for 24 h or less and failed to stimulate an increase in plasma concentrations of bST (13, 26). Cohick et al. (7) also did not detect changes in plasma concentrations of bST, insulin, prolactin, triiodothyronine, or thyroxine when casein was infused continuously into the abomasum, but milk production increased significantly. Those authors suggested that postruminal protein infusion may increase milk production by increasing gluconeogenesis because plasma concentrations of glucagon were increased significantly.

Plasma concentrations of bST are positively and significantly correlated with milk production (3, 16). Milk production was not significantly increased in our trial even though small increases were observed in plasma bST concentrations when arginine was injected into the jugular vein of lactating cows. These data suggest that if manipulations of plasma concentrations of endogenous bST are to result in increased milk production, the manipulations should be specific for bST and should produce a large and prolonged release of bST. The rapid rate at which arginine and ornithine are catabolized may prevent these two metabolites from effectively being used to increase plasma bST concentrations in dairy cows.

REFERENCES

by arginine infusion. Endocrinology 103:1752.