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Original article

Effect of abomasal infusion of aspartate on nitrogen balance and plasma amino acids in Holstein steers*

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Abstract – We investigated the effect of abomasally infused aspartate (Asp) on N balance and plasma amino acids in steers. Four ruminally cannulated Holstein steers (180 kg) housed in metabolism crates were used in an experiment designed as a 4 × 3 Youden square. Steers received continuous abomasal infusions of water or water containing 40 or 80 g Asp/d. Steers were fed twice daily a diet containing 473 g/kg corn, 463 g/kg alfalfa hay and 52 g/kg soybean meal at levels near ad libitum intake. Abomasally infused Asp had no effect on N balance. Infusion of 80 g Asp/d increased ($P < 0.05$) plasma concentrations of Asp, glutamate and alanine. Metabolism of Asp by gut tissues probably prevented the large change in plasma concentration of Asp that seems necessary to trigger hormonal responses. We conclude that abomasal supplementation of steers with up to 80 g/d of Asp does not enhance performance. © Inra/Elsevier, Paris

cattle / nitrogen balance / aspartate / amino acid

Résumé – Effet d'une infusion abomasale d'aspartate sur le bilan azoté et les acides aminés plasmatiques chez les taurillons Holstein. L'effet d'une infusion abomasale d'aspartate (Asp) sur le bilan azoté et les acides aminés plasmatiques a été étudié chez des taurillons. Quatre taurillons Holstein de 180 kg munis d'une canule du rumen et maintenus dans des cages métaboliques ont été utilisés dans une expérience en 4 × 3 carré de Younden. Les animaux recevaient en continu des infusions abomasales d'eau seule ou d'eau apportant 40 ou 80 g d'aspartate par jour. Ils consommaient deux fois par jour un régime, 473g/kg de maïs, 463 g/kg de foin de luzerne et 52 g/kg de tourteau de soja à des niveaux proches de ad libitum. Les infusions d'aspartate n'ont pas eu d'effet sur le bilan azoté. L'infusion de 80 g Asp par jour augmentait ($P < 0,05$), les concentrations plasmatiques de Asp, glutamate et alanine. Il est probable que la métabolisation de l'aspartate par la membrane intestinale a empêché une forte

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modification de la teneur en Asp du plasma qui semble nécessaire pour déclencher des réponses hormonales. En conclusion, un supplément abomasal de 80 g d'aspartate par jour chez des taurillons n'augmente pas les performances. © Inra/Elsevier, Paris

bovin / bilan azoté / aspartate / amino-acide

1. INTRODUCTION

Consumer resistance to the use of exogenous hormones to enhance animal productivity has prompted attempts to manipulate endogenous hormone supplies. Growth hormone especially has been targeted because it is involved intimately with the control of nutrient partitioning, lactation and growth. Research with rats and sheep has shown that aspartate (Asp) infused intravenously is a strong growth hormone secretagogue [1, 3]. Although duodenal [4] and abomasal [9] infusions of Asp failed to stimulate growth hormone secretion in wethers, Asp did increase secretion of glucagon, insulin and IGF-1 [4]. A rise in circulating IGF-1 may stimulate growth independently of a growth hormone response [8]. However, performance studies conducted with Asp, as well as studies with cattle, are lacking. Our objective was to determine the effects of graded levels of Asp administered abomasally on N balance and plasma amino acids in Holstein steers.

2. MATERIALS AND METHODS

Four ruminally cannulated Holstein steers (180 ± 7 kg) were used in an experiment designed as a 4 × 3 Youden square. Steers were adapted to the experimental diet before the start of the trial. Each of the three periods lasted 6 d: 2 d for adaptation to infusions and 4 d for sample collection. A short adaptation period was appropriate because N status of ruminants adapts rapidly to postruminally infused treatments [2]. Treatments consisted of 4 L of water only or water containing 40 or 80 g L-Asp/d and were infused continuously into the abomasum over a 24-h period. To aid in solubilization, 24 g of NaOH (equal on a molar basis to 80 g Asp) were added to the water prior to addition of the 80 g of Asp. An equal amount of NaOH was added to the 0 and 40 g/d infusates to maintain isosodium conditions between treatments and acetic acid (equimolar to the excess NaOH) was added to neutralize the NaOH. Final pH of all infusates was approximately 6. Treatments were infused by a peristaltic pump with polyvinyl chloride tubing passed through the ruminal cannula and into the abomasum via the reticulo-omasal orifice; the tubing was held in the abomasum by a small rubber flange. Steers were housed in metabolism crates in an environmentally controlled room (constant temperature) with continuous lighting. They were fed at a level slightly less than ad libitum intake (constant for each animal for the duration of the experiment) and had free access to water. Each steer received its daily allotment of feed in two equal portions (0600 and 1800 hours). The diet (*table I*) contained 158 g/kg crude protein and was designed to exceed the steers' protein needs. Any feed refusals were collected each morning before the morning feeding, weighed and stored in a freezer.

During each 4-d phase of sampling, total excreta was collected. Urine was collected into recepticles containing enough 6 N HCl to ensure that urinary pH was below 3. One-tenth (by weight) of daily urinary output was composited and frozen for N analysis. Similarly, one-tenth of fecal output was composited and frozen daily.

A sample of jugular blood was collected from each steer into heparinized vacutainer tubes approximately 5 h after the morning feeding on the last day of every period. Samples were cooled immediately in ice water and then centrifuged at 4 °C for 20 min at 5 000 *g*. Equal amounts of plasma and 100 g/L sulfosalicylic acid, containing norleucine (1 mM) as an internal standard, were mixed and cooled on ice for 30 min. Subsequently, the samples were cen-

trifuged at 31 000 *g* for 20 min and the supernatant was frozen at –20 °C pending later analysis of amino acids.

Ort samples were dried in a forced-air oven at 50 °C for 48 h. Feed and ort samples were ground to pass through a 1-mm screen and were analysed for DM (100 °C for 24 h) and Kjeldahl N. Urine and wet fecal samples were analysed for Kjeldahl N. Plasma amino acids were separated by cation exchange chromatography and measured via fluorimetry following postcolumn *o*-phthalaldeh yde derivitization (Beckman System Gold; Beckman Inc., Palo Alto, CA, USA).

Data were analysed statistically by analysis of variance using the general linear models procedure of SAS [7]. The model included effects of steer, period and treatment.

3. RESULTS AND DISCUSSION

Dry matter consumption averaged 5.53 ± 0.05 kg/d. Fecal N excretion did not increase (*table II*) when Asp was infused, suggesting that it was absorbed efficiently. Urinary N excretion tended to increase linearly with increasing levels of Asp. Consequently, abomasally infused Asp did not affect N retention.

Plasma concentration of Asp (*table III*) was not increased by 40 g Asp/d, but was increased ($P < 0.05$) by 80 g Asp/d (from 16 to 23 μM). Infusion of 80 g Asp/d also increased ($P < 0.05$) plasma concentrations of glutamate and alanine. Plasma concentrations of tyrosine and methionine decreased with 40 g Asp/d, but remained unchanged for the 80 g/d treatment.

Endocrine responses to amino acid challenges are often complex and difficult to describe. For example, in sheep, Asp has increased growth hormone secretion without affecting IGF-1 concentra-

Table I. Ingredient and nutrient composition of the diet.

Item	g/kg
Ingredient	(dry matter basis)
Rolled corn	473
Alfalfa hay	463
Soybean meal	52
Dicalcium phosphate[a]	11
Vitamins ADE[b]	1
Nutrient	
Crude protein	158.4
Ca	8.8
P	5.5
K	10.9

[a] Contained 180 g Ca/kg and 210 g P/kg.
[b] Provided 4 400 IU of vitamin A, 2 200 IU of vitamin D, and 13.2 IU of vitamin E per kg of diet dry matter.

Table II. Effect of abomasal infusion of graded levels of aspartate on N balance in growing Holstein steers.

Nitrogen	Aspartate (g/d)			SEM
	0	40	80	
	g N/d			
Feed	141.7	138.8	138.8	1.3
Infused[a]	0	4.2	8.5	0
Fecal	46.6	44.7	45.2	1.2
Urinary	61.7	67.6	68.9	4.7
Retained	33.5	30.7	33.2	5.0

[a] Nitrogen contained in aspartate.

tion [3] and also has increased IGF-1 concentration without affecting growth hormone secretion [9]. In another study with sheep, Asp failed to produce a growth hormone response but did increase plasma concentrations of glucagon, insulin and glucose [4]. Because performance studies are needed before any biological significance can be attached to manipulation of the endocrine system by using neuroexcitatory amino acids such as Asp, we chose to use N retention (a measure of lean tissue deposition), rather than hormone concentrations, as the primary response criterion for our experiment.

A large and rapid change in plasma concentration of a neuroexcitatory amino acid seems necessary to elicit a growth hormone response. Kuhara et al. [3] infused wethers intravenously with 399 mg Asp/kg BW over a 30-min interval (13.3 mg per kg BW/min) and observed large increases in growth hormone concentrations. However, when the same dose was infused abomasally over a 24-h period (0.3 mg per kg BW/min), no growth hormone response was measured [9].

In contrast to the almost instantaneous change in plasma concentration of an amino acid caused by intravenous infusion, various physiological constraints limit manipulation of plasma concentration of Asp by gastric supplementation. Absorption of Asp across the gut wall

Table III. Plasma concentrations of amino acids in steers infused abomasally with aspartate.

Amino acid	Aspartate (g/d)			SEM
	0	40	80	
	μM			
Lysine	59.3	55.0	60.7	5.7
Methionine	25.0[ab]	23.6[a]	25.7[b]	.5
Histidine	52.1	49.5	49.3	2.6
Arginine	110.2	86.4	102.0	9.1
Threonine	66.8	57.2	58.4	3.9
Valine	177.9	156.6	182.5	15.0
Isoleucine	91.0	83.1	97.1	6.0
Leucine	113.8	106.1	121.6	6.7
Tyrosine	59.9[a]	50.3[b]	61.7[a]	2.0
Phenylalanine	49.8	41.6	47.4	1.5
Aspartic acid	16.0[a]	16.3[a]	23.0[b]	.8
Serine	76.6	70.9	81.5	3.3
Glutamic acid	96.1[a]	88.5[a]	115.6[b]	4.0
Glycine	221.0	229.0	220.1	12.8
Alanine	157.4[a]	165.2[a]	195.9[b]	4.1
Asparagine	23.7	23.7	23.9	2.6
Glutamine	210.3	258.1	218.6	16.6
Citrulline	57.7	54.5	52.3	2.4
Taurine	11.8	14.5	12.7	1.0
Ornithine	56.2	66.6	60.9	7.0
Total	1732.5	1696.6	1810.8	60.9

[a,b] Means not bearing common superscripts differ, $P < 0.05$.

poses the first constraint, because luminal Asp is an important metabolic fuel for gut tissues [10]. When animals are fed conventional diets, absorbed Asp seldom reaches the periferal circulation, because it is metabolized extensively by the gut wall. Thus, net output of Asp by portal-drained viscera is small in comparison with that of most other amino acids, with the exception of glutamine and glutamate [11]. Prior et al. [6] reported that, for steers (270 kg) fed 4.4 kg of a corn-based diet, gut tissues utilized more Asp than the amount absorbed from the gut.

Given these constraints, a large dose of dietary Asp may be necessary to elicit hormonal responses. We estimated that the basal supply of absorbable Asp in our study was approximately 40 g/d. We chose the levels of infusion to raise the supply of Asp to two and three times this amount with the intention of exceeding gut capacity to metabolize it. A dose and infusion rate of Asp similar to our 80 g/d level (0.3 mg Asp per kg BW/min) increased circulating IGF-1, glucagon and insulin in wethers [9].

Plasma concentration of Asp was not increased by 40 g Asp/d in our study, suggesting that either the capacity of gut tissues to metabolize Asp was not exceeded or that body tissues were able to utilize Asp that escaped gut metabolism. The 80 g/d treatment did alter plasma concentration of Asp, but the change was relatively small. The rise in plasma concentration of alanine (*table III*) with increased abomasal infusion of Asp in our study is consistent with the ability of gut mucosa to transaminate Asp to alanine [5]. The increased concentration of plasma glutamate may have stemmed, in turn, from hepatic uptake of alanine and subsequent conversion to glutamate [11]. Increased plasma concentration of alanine arguably could be responsible for some of the reported endocrinological changes observed with Asp administration. However, as for Asp, the threshold level at which plasma alanine can cause pharmacological actions probably was not exceeded in our study.

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