Nutritional Requirements of *Allisonella histaminiformans*, a Ruminal Bacterium that Decarboxylates Histidine and Produces Histamine

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Abstract. Histamine is an inflammatory agent that contributes to bovine laminitis. Cattle fed silage-containing rations often have large populations of *Allisonella histaminiformans*, but this obligate histidine-decarboxylating bacterium could not be isolated from cattle fed timothy hay. The growth of *A. histaminiformans* was stimulated by yeast extract, protein hydrolysates, and water-soluble extracts of alfalfa or corn silage. Extracts of alfalfa were more potent than corn silage. Because growth and histamine production were not stimulated by Casamino Acids or a mixture of purified amino acids, it appeared that *A. histaminiformans* requires peptides. The idea that *A. histaminiformans* requires peptides is consistent with the observation that alfalfa silages often have a large amount of peptide nitrogen.

It has long been recognized that bacteria can decarboxylate amino acids [8]. Histidine decarboxylation produces histamine, an amine with strong biogenic properties [23]; and foods contaminated with histamine-producing bacteria are a serious human health problem [4, 11, 14]. Histamine can also be produced in the rumen, and in the 1950s, Dougherty and his colleagues concluded that there was a direct negative correlation between “the histamine level of ingesta and the well being of the animal” [6]. Tissues above the hoof of ungulates and equids are particularly sensitive to histamine, and animals with elevated serum histamine often have sore feet (laminitis) [21, 22]. Laminitis has a serious negative impact on the economics of dairy farming; Nelson and Cattell [18] noted that cows with a history of laminitis lesions were culled during the current lactation at a rate 1.77 times higher than cows with no history of laminitis lesions.” These same authors also noted “a history of laminitis increased the likelihood of culling due to reproduction, mastitis, low production and other reasons.”

In the 1950s, Rodwell [19] isolated histidine-decarboxylating lactobacilli from sheep and horses. It had generally been assumed that lactobacilli were responsible for much of the ruminal histamine accumulation [10], but recent work indicated that a highly specialized and previously unrecognized bacterium, *Allisonella histaminiformans*, seemed to be more important for ruminal histamine accumulation [9]. *A. histaminiformans* is a low G+C (firmicutes) bacterium that grows rapidly, catabolizes histidine as its sole energy source for growth, and produces histamine in a 1:1 ratio of histidine consumption [9].

Previous work indicated that *A. histaminiformans* could be readily isolated from the rumen of cattle fed a commercial dairy ration but not in cattle fed only timothy hay [10]. Cattle fed the commercial dairy ration had a lower ruminal pH than those fed hay, but the difference was less than 0.4 pH units (6.3 versus 6.7). The dairy ration contained more crude protein than the hay, but once again the difference was small (18% versus 14%). Because preliminary experiments indicated that the diet-dependent difference in *A. histaminiformans* numbers could be manifested in the same cow (data not shown), it appeared that animal variability was not the only factor involved. The following experiments were designed to define more precisely the nutritional requirements of *A. histaminiformans*.

Materials and Methods

Diet, animals, and ruminal fluid. Ruminal contents were obtained from fistulated, non-pregnant, non-lactating dairy cows (Institutional...
Animal Care and Use Committee (IACUC) protocol #95-1-00) fed a diet of timothy hay (10 kg dry matter per day, 14% crude protein, 40% neutral detergent fiber). Ruminal fluid (1000 mL) was brought to the laboratory and placed in a 39°C water bath. After gas production from fermentation had buoyed small feed particles to the top of the flask and protozoa sedimented to the bottom, mixed ruminal bacteria were withdrawn from the center of the flask.

**Culture conditions.** *A. histaminiformans* MR2T (ATCC BAA610, type strain) was routinely cultivated in basal medium that contained (per liter): 50 mmol histidine, 10 mmol butyric acid, 292 mg K2HPO4, 480 mg (NH4)2SO4, 480 mg NaCl, 100 mg MgSO4 · 7H2O, 64 mg CaCl2 · 2H2O, 500 mg cysteine hydrochloride, 500 mg yeast extract and 4 g Na2CO3 (pH 6.0). In some cases, the yeast extract was replaced with a vitamin mixture that supplied (per liter): 1 mg pyridoxamine 2HCl, 2 mg riboflavin, 2 mg thiamine HCl, 2 mg nicotinamide, 2 mg CaD pantothenate, 1 mg thiotic acid, 0.1 mg p-aminobenzoic acid, 0.05 mg folic acid, 0.05 mg biotin, 0.05 mg cobalamine, 1 mg pyridoxal HCl and 1 mg pyridoxine. The purified amino acid mixture supplied an equal part mixture of the 20 amino acids commonly found in protein (12 mg/mL total amino acid concentration). In some cases, the yeast extract was deleted and Casamino Acids, Trypticase, Bacto Peptone (Difco, Sparks, MD), gelatin hydrolysate, soybean protein hydrolysate, or lactalbumin hydrolysate (U.S. Biochemical, Cleveland, OH) were added. *Lactobacillus 30A* was grown on MRS medium [7] plus 50 mM histidine. Bacteria were anaerobically cultivated (39°C) in 18 × 150 mm tubes that were capped with butyl rubber stoppers and aluminum seals. Growth was monitored via changes in optical density (1 cm cuvette, 600 nm, Gilford 260 spectrophotometer). *A. histaminiformans* MR2T was serially diluted (10-fold increments) into basal medium or sterile rumen fluid in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, MI) and the tubes (1.8 mL, 13 × 100 mm) were capped and incubated at 39°C.

**Sterile ruminal fluid.** Ruminal fluid was clarified by centrifugation (10,000 g, 15 min, 5°C, 3 times). The clarified ruminal fluid (50 mL) was then dispensed anaerobically into bottles, flushed with oxygen-free CO2 and autoclaved (121°C, 20 min). Once the bottle had cooled to room temperature, histidine (50 mM) was added, and the sealed bottles were transported into the anaerobic glove box.

**Histamine determination.** Histamine was assayed via a method that employed thin-layer chromatography [12]. Cell-free culture supernatant samples (10 μL) were applied to TLC plates (Merck Art. 5737 silica gel 60/kieselguhr F254 pre-coated, layer thickness 0.25 mm). The plates were immersed to a depth of 1 cm in solvent (20 parts methanol and 1 part ammonium hydroxide, v/v) for 90 min. The plates were removed from the solvent chamber and placed in an oven (70°C) for 15 min. The plates were then sprayed immediately with ninhydrin (3% w/vol in methanol).

**Silage and feed extracts.** Extracts were prepared from alfalfa silage, fresh alfalfa, corn silage, fresh chopped corn, and high-moisture shelled corn. Samples (18 g dry matter) were extracted with water (300 mL) using a blender (14 speed Blendmaster Ultra, Hamilton Beach, Washington, NC) at the highest setting for 2.5 min. The pulverized materials were strained through two layers of cheesecloth. The liquid extracts were boiled under N2 and transferred anaerobically to tubes that were capped with butyl rubber stoppers, and sealed with aluminum caps. The extracts were autoclaved (121°C, 15 min) or sterile filtered (0.2 μm, Corning Inc., Corning, NY) and added to basal medium lacking yeast extract (typically 0.4 mL into 10 mL or 2.4 mg extract dry matter per mL of starting material). Non-ensiled feeds and silages (30 g) were dried in an oven (110°C, 12 h) to determine dry matter [11].

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**Results**

Preliminary experiments indicated that thin-layer chromatography could be used as a method for separating, detecting, and quantifying histidine and histamine (Fig. 1). When stationary phase *A. histaminiformans* MR2T cultures (10⁹ viable cells/mL) were serially diluted (10-fold increments) into ruminal fluid from cows fed timothy hay that had been clarified, autoclaved, and supplemented with histidine (50 mM) and (b) ruminal fluid supplemented with histidine (50 mM) and yeast extract (0.6 mg/mL).

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![Fig. 1. A thin-layer chromatograph showing histidine and histamine standards prepared in water. The histidine and histamine concentrations were 50 mM (a), 25 mM (b), 10 mM (c), 5 mM (d), and 2.5 mM (e). Basal medium lacking histidine (f) and clarified ruminal fluid from a cow fed timothy hay (g) are also shown.](image-url)