

## Stability of GAA and creatine monohydrate in dry and canned canine diets

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**Introduction:** Creatine (Cr) is a naturally occurring substance in animals and animal derived proteins and acts in its phosphorylated form PCr as energy buffer in muscle cells. The synthetic product creatine monohydrate (CM) is widely used as a dietary supplement for humans. Limitations are noticed in the use as a direct animal feed additive due to instability of CM in aqueous solution and high temperature, where cyclic creatinine is formed which is physiologically inactive. Guanidinoacetic acid (GAA) is the direct metabolic precursor of creatine. The objective of this study was to examine the in-feed mixing uniformity, heat-stability (extrusion or can retorting) and storage stability of GAA and creatine monohydrate (CM) in a dry (extruded) and wet (canned) canine diet.

**Materials and methods:** Isomolar levels of GAA (2.2 g/kg) and creatine from CM (2.8 g/kg CM = 2.46 g/kg creatine) were mixed in a dry canine diet (pre-mixture: Forberg F60 paddle shift mixer, 3 min; final mixture: Vrieco vertical mixer, 15 min), extruded (conditioning: 8% water/3% steam at 100°C, 25 sec; extrusion: 100-130 °C, 10 sec; Almex AL150), dried (50 °C overnight), packed and stored (25°C/60 % RH/ 15 months and 40°C/75 % RH/ 6 months). Mixing uniformity (MU) and extrusion stability (ES) were determined in 10 samples each.

For the canning study, 1 g/kg GAA and 1 g/kg CM (= 0.879 g creatine) were each mixed (Bestron mixer; 2 min) to a commercial wet (paté-like) diet (basis: meat/animal derivatives) to examine MU, canned, sealed and heat-sterilized (HSS, 127 °C, 60 min) in a batch retorting system. At each step, 8 samples were taken for analysis. Storage stability (SS) of the canned diets was determined as for the dry foods. The wet diet contained 810 g/kg water at pH 5.8. Results on the CM-diets were corrected for blank values in the base diets (dry diet: creatine: 107 mg/kg, creatinine: 831 mg/kg; wet diet: 276.5 mg/kg creatine, 7.8 mg/kg creatinine).

**Results and discussion:** All absolute values given on as-is basis, recovery calculated on DM. Dry food study: Mean MU was  $2.01 \pm 0.13$  g/kg, for GAA (initial value 2.2 g/kg) and  $2.64 \pm 0.06$  for creatine in CM (initial value 2.46 g/kg). Mean ES was  $2.23 \pm 0.09$  g/kg for GAA and  $2.28 \pm 0.01$  g/kg for CM, which gives a recovery of 101.4 % and 84.6 % for GAA and CM respectively. Recovery upon storage for GAA was between 95 and 100 % for all samples stored at 25 or 40°C. Creatine content in the CM diets steadily decreased with time and the loss was found almost completely as creatinine. Storage recovery of CM, was 46 % after 15 months/25 °C and 33 % after 6 months/40°C.

Data for the canned food study are given in Table 1.

Table 1. Recovery for GAA and CM during the manufacturing of a wet (canned) canine diet

	Suppl. (mg/kg food)	MU (mg/kg food)	HSS (mg/kg food)	Recov.HSS (%)	SS (40 °C/6 m) (mg/kg food)
GAA	1000	987.1 +/-39.8	895.9 +/-28.0	84.5	869
CM (base corr).	879	893.9 +/-38.0	321.0 +/-19.0	37.3	444

Creatine in CM-diets decreased drastically upon sterilisation but increased again with storage.

**Conclusion:** In dry diets, mixing and extrusion stability was excellent for GAA, whereas CM stability was adequate. Storage stability remained excellent for GAA but was poor for CM. In wet, canned diets, GAA had good mixing uniformity and sterilization stability. For CM, a massive transition of creatine to creatinine was observed during sterilization. Because in aqueous solution, creatinine can be retransformed to creatine, creatine assay increased again in the wet diets during storage.