Short-term effects of dietary supplementation with amino acids in dogs with proteinuric chronic kidney disease

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Abstract: This retrospective study investigated the impact of amino acid supplementation on body weight, serum albumin, creatinine and urea concentrations, and urine protein-to-creatinine (UPC) ratio in proteinuric dogs with chronic kidney disease (CKD). Forty-six client-owned azotemic dogs with spontaneous proteinuric CKD already on a renal diet and in therapy with enalapril were included. After approximately 1 month of treatment (baseline), 29 dogs received oral amino acid supplementation daily (group A) and 17 dogs did not (group B). The parameters under investigation were determined at baseline and after 4 to 8 weeks in both groups. Compared to baseline, body weight and serum albumin increased ($P < 0.01$, $P < 0.05$, respectively) at follow-up in group A, but did not change in group B. Serum creatinine concentration did not change in both groups; urea concentration ($P < 0.05$) and UPC ratio ($P < 0.01$) decreased in group B, but not in group A. Supplementation with amino acids increased body weight and serum albumin concentration in these dogs but it might have prevented a decrease in proteinuria and urea concentration.

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SHORT-TERM EFFECTS OF DIETARY SUPPLEMENTATION WITH AMINO ACIDS IN DOGS WITH PROTEINURIC CHRONIC KIDNEY DISEASE

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Abstract

As malnourishment in humans with chronic kidney disease (CKD) is associated with increased morbidity and mortality, oral supplementation with amino acids is proposed. In dogs, malnutrition may be associated with CKD and increases the rate of renal-related complication and mortality. Aim of this retrospective study was to verify the impact of amino acid supplementation on body weight, serum albumin, creatinine and urea concentrations, and urine protein-to-creatinine (UPC) ratio in proteinuric CKD dogs. Forty-six client-owned azotemic dogs with spontaneous proteinuric CKD already on a renal diet and in therapy with enalapril were included. After approximately one month of treatment (baseline), 29 dogs received oral amino acid supplementation daily (group A), whereas 17 dogs did not (group B). The amount of amino acids was calculated as follows: body weight (kg) × [UPC ratio] × 20 = X , where X are the milligrams of amino acids to be administered daily. Body weight, serum albumin, creatinine and urea concentrations, as well as the UPC ratio were determined at baseline and after 4-8 weeks in both groups. Compared to baseline, body weight increased ($P<0.01$) at follow-up in group A, while it did not change in group B. Serum albumin concentration increased ($P<0.05$) in group A and it did not change in group B. Serum creatinine concentration did not vary in both groups; urea concentration ($P<0.05$) and UPC ratio ($P<0.01$) decreased in group B, but not in group A. Although supplementation with amino acids increased body weight and serum albumin concentration in dogs, maintaining stable serum creatinine concentration, it might have **prevented a decrease of proteinuria and of urea**. Amino acid supplementation may be considered in the treatment of proteinuric dogs, if hypoalbuminemia is severe and antiproteinuric therapy is not effective.

Abbreviations: amino acids, AA; angiotensin converting enzyme inhibitors, ACEI; borderline proteinuria, BP; body weight, BW; chronic kidney disease, CKD; dry matter, DM; International Renal Interest Society, IRIS; non-proteinuric, NP; proteinuric, P; urine protein-to-creatinine, UPC.

Introduction

In dogs, proteinuria is often associated with chronic kidney disease (CKD) and studies in this species led to hypothesize that it may promote the progression of renal damage, as it happens in humans (1-8). In endemic areas for vector-borne diseases, such as leishmaniosis, the prevalence of dogs with proteinuria, azotemia, or both, has been reported to be up to 50% (8,9). Among dogs at risk for developing proteinuric nephropathy, other than those living or having lived in endemic areas, there are also breeds that are genetically predisposed to proteinuric CKD (3,4,6,8). Early identification and treatment of proteinuria appears to be crucial in dogs, not only because of the high prevalence, but also as its management slows the progression of renal disease, risk of uremic crisis and renal-related death (1,3,4). Together with the treatment of the underlying disease, the major cornerstones of therapy in proteinuric CKD dogs are angiotensin converting enzyme inhibitors (ACEI), dietary intervention and **omega-3** fatty acids which proved to slow the progression of renal disease, minimize clinical signs of uremia and, at least for the diet, to maintain either an optimal body weight (BW) and body condition score (1-3,6,9-14). However, in some dogs, anti-proteinuric therapy may not reduce proteinuria **despite diets lower in protein compared to maintenance diets** are **administered concurrently** (2-4,8-11,13,14). Furthermore, in proteinuric dogs renal diets may not adequately meet protein requirements, thus possibly leading to low BW, hypoalbuminemia and malnutrition. A previously published study on a spontaneous model of proteinuric nephropathy in dogs, showed that a low protein content diet (14% on dry matter basis), similar to the renal diets commonly recommended in dogs with kidney disease, caused a significant reduction in BW and plasma albumin concentration that were already noticeable at 4 weeks of administration (8).

In humans with CKD, nutritional status is helpful to identify patients with increased risk of morbidity and mortality; a significant association, indeed, was observed between decreased baseline BW condition and subsequent risk of hospital admission (15-18). For these reasons, in

73 malnourished humans with CKD oral supplementation with amino acids (AA) or intradialytic AA
74 administration have been proposed (15-19).
75 In CKD dogs with severe proteinuria , either low BW and hypoalbuminemia are frequent and they
76 can be associated with increased morbidity and risk of mortality (20). Indeed, albumin
77 hypercatabolism and its down-regulated synthesis can contribute to glomerular disease-associated
78 hypoalbuminemia, possibly leading to marked hypoalbuminemia in CKD dogs with sub-nephrotic
79 range proteinuria thus worsening the prognosis (21). Based on this premise, it seems plausible that
80 the amount of proteins needed should be individually-tailored in dogs, depending on the stage of
81 CKD and the entity of proteinuria (8). As in humans, also in dogs AA supplementation may
82 represent the easiest means to correct an insufficient daily intake of proteins. Thus, the aim of this
83 retrospective case-control study was to investigate the impact of an oral AA supplementation during
84 a short period of time on BW, serum concentrations of albumin, creatinine and urea, and on the
85 urine protein-to-creatinine (UPC) ratio in proteinuric CKD dogs treated with enalapril and fed a
86 commercial renal diet (RD).
87

Materials and Methods

Animals and inclusion criteria

Medical records of proteinuric dogs in IRIS stages ≥2 (14) admitted in 2007 and 2008 at one of the authors' institutions (AZ, PDI) were reviewed. All the data available regarding clinical history, physical examination, BW, complete blood count, serum biochemical profile, urinalysis, UPC ratio, indirect blood pressure measurements, abdominal ultrasonographic findings, ongoing treatments and follow-up examinations were collected. Dogs without stable renal function were excluded; stable renal function was defined by serum creatinine concentration that did not increase or decrease by 20% or more within one month from initial determination (10). Dogs were considered to be proteinuric if the UPC ratio was above 0.5 (IRIS substage P) (14) in two urine samples collected at one-month interval; dogs that did not fulfil this criterion were excluded. Furthermore, to be included in the study, dogs had to receive enalapril (Enacard®, Merial Italia spa, Milano, Italy) at 0.5 mg/kg (0.23 mg/lb), q 24 h, and a commercial renal diet (Hill's Prescription Diet Canine k/d®, Hill's Pet Nutrition Inc., Topeka, KS or Royal Canin Renal Canine®, Royal Canin SA, Aimargues, France); **the amount of diet was according to the suggestions of the companies and corrections were not performed if dogs received or not AA supplementation.**

From each record, information was collected to identify dogs that received or not oral AA supplementation (IT IS pet, ACME srl, Cavriago (RE), Italy; formulation available in table 1). Among dogs on AA supplementation, only those taking the daily amount (mg) of AA arbitrarily calculated using the following formula, BW (kg) × [UPC ratio] × 20 = X (22), where X are the milligrams of amino acids to be administered daily were included. Based on the product indications, 1 tablet provides approximately 675 mg of AA. Dogs that had received oral or intravenous AA supplementation within one month from time of admission were excluded. Finally, dogs were excluded if the diagnostic workup identified any inflammations or infections of the genitourinary tract (based on ultrasonography and urinalysis), a pre-renal cause of proteinuria (based on serum biochemistry), and if cardiac disease, neoplasia or endocrinopathies were diagnosed or suspected.

All dogs had been tested for leishmaniosis, ehrlichiosis and babesiosis, and were not included if an active form of infection was either identified or suspected.

Additional treatments and follow-up

As a standard of care at the authors' institution, dogs classified as "severely hypertensive" (systolic arterial pressure ≥180 mm Hg) accordingly to the IRIS staging system (14) were treated with oral amlodipine at 0.1 to 0.5 mg/kg (0.05 to 0.23 mg/lb), q 24 h, in order to reduce systolic arterial pressure to <160 mm Hg (substage "normotensive" or "borderline hypertensive"). In addition, dogs with severe hypoalbuminemia received oral acetylsalicylic acid at 2.0 mg/kg (0.91 mg/lb), q 24 h, to prevent thrombosis. Based on the reference range of serum albumin (2.8 to 3.8 g/dL), dogs were considered hypoalbuminemic if the albumin concentration was ≤2.7 g/dL; severe hypoalbuminemia was arbitrarily defined as a value <2.0 g/dL.

As stated above, dogs with proteinuric CKD were reassessed after one month to check if the renal disease was stable; all throughout the manuscript this time-point will be called *baseline*. After baseline, dogs were re-evaluated between 4-8 weeks.

Blood sampling and assay

During each examination, blood samples were collected in dogs fasted overnight, and serum was obtained within 30 minutes, stored at 4 °C (39 °F) and analyzed within 24 hours. Results from complete blood count and serum biochemical analysis, including albumin, total proteins, glucose, bilirubin, cholesterol, amylase, alanine transferase, alkaline phosphatase, urea nitrogen, creatinine, sodium, potassium, chloride and phosphate, were achieved with the same methods (BC-2800Vet, MINDRAY, Mindray Co. Ltd., Shenzhen, China; Cobas Mira, Roche Diagnostic AG, Basel, Switzerland) in all samples.

Urine collection and urinalysis

An ultrasound-guided cystocentesis was performed in all dogs using a 5 mL syringe connected to a 23-gauge needle. All urine samples were placed in 10 mL, sterile, evacuated collection tubes, and analyzed by the same operator. **Urine samples** were examined within 60 minutes from collection if samples were stored at room temperature (approximately 20 °C [676 °F]), or within 4 hours if stored at 4 to 8 °C (135 to 270 °F). Urine sediment was obtained by centrifugation (10 minutes at 900 × *g*) of 5 mL of urine, followed by removal of 4.5 mL of supernatant, and resuspension of the remaining 0.5 mL of urine. A sample of 12 µL of the resuspended urine was microscopically assessed. The supernatant was transferred into separate tubes and stored at –20 °C (676 °F) to determine UPC ratio within 7 days. Red blood cells and white blood cells were expressed as mean number of cells/10 hpf (40 × magnification). Urine sediment with bacteriuria, and/or >5 red blood cells or white blood cells/hpf, was considered indicative of active inflammation and excluded from the UPC ratio evaluation (23).

UPC ratio

To calculate the UPC ratio, protein concentration (mg/dL) was measured with pyrogallol red, and creatinine (mg/dL) was measured using the Jaffé method on undiluted urine supernatant that was thawed before analysis. Analytes were measured in an automated spectrophotometer (Cobas Mira, Roche Diagnostic AG, Basel, Switzerland) in each case.

Statistical analysis

For data evaluation, BW, serum albumin, creatinine and urea concentrations, and the UPC ratio were retrieved from baseline and after 4-8 weeks in all dogs. Dogs that received the AA supplementation were included in the group A, those that did not receive the AA supplementation belonged to the group B (no placebo was provided). To verify whether population characteristics were similar in the two groups, baseline age, BW, serum albumin, creatinine and urea

concentrations, and UPC ratio were compared with unpaired t-test. Sex distribution, and frequency and severity of hypoalbuminemia were compared between groups with chi-squared test or Fisher's exact test.

To study the effect of AA supplementation, BW, serum albumin, creatinine and urea concentrations, and UPC ratio at baseline and after 4-8 weeks were compared within each group with paired t-test. Because severe hypoalbuminemia may be associated with morbidity and mortality in dogs (3,20,21), the effect of AA supplementation was also explored in the subset of cases with serum albumin concentration <2.0 g/dL by paired comparisons between baseline and 4-8 weeks for the above parameters. Normality of all data sets was investigated with Kolmogorov-Smirnov test and non-normally distributed variables were log-transformed to achieve Gaussian distribution prior to using parametric tests. Results are reported as mean ± standard deviation or as percentages. A $P<0.05$ was considered statistically significant. Statistical analysis was performed with commercial software (GraphPad Prism version 4.0, GraphPad Software Inc., La Jolla, CA).

Results

Baseline

Forty-six proteinuric CKD dogs in IRIS stages 2, 3 or 4 were included; 29 of them received AA supplementation (group A), while the remaining 17 did not (group B).

Age and BW of both groups are reported in table 2. In group A, 20 (69%) dogs were males (17 intact and 3 castrated) and 9 (31%) were females (8 intact and 1 spayed). Regarding dog breeds, 7 were Boxer, 2 of each were German Shepherd, Dogue de Bordeaux, Epagneul Breton or Italian Pointer, and one of each was Cocker Spaniel, Dachshund, Dalmatian, Doberman, Dogo Argentino, German Pointer, Golden Retriever, Jack Russel Terrier, Pitt Bull and Rottweiler; the remaining 4 dogs were cross-breed. In group B, 11 (64.7%) dogs were intact males and 6 (35.3%) were females (5 intact and 1 spayed). Regarding dog breeds, 3 were Boxer, 2 were Great Dane, and one of each was American Staffordshire, Dalmatian, Dogo Argentino, Dogue de Bordeaux, English Setter, German Pointer, German Shepherd, Irish Wolfhound, Labrador and Pomeranian; the remaining 2 dogs were cross-breed. Age, sex distribution and BW did not significantly differ between groups.

Serum concentration of creatinine, urea and albumin, as well as UPC ratio of either group are reported in table 2. In group A, 18 (62.1%) dogs were in IRIS stage 2, 9 (31.0%) in IRIS stage 3 and 2 (6.9%) were in IRIS stage 4. In group B, 7 (41.2%) dogs were in IRIS stage 2, 5 (29.4%) in IRIS stage 3 and 5 (29.4%) in IRIS stage 4. Serum concentration of creatinine was significantly lower in dogs in group A ($P<0.05$), whereas albumin and urea, and the UPC ratio did not significantly differ between groups. In group A, 20 (69.0%) dogs had low albumin concentration, 11 of which showing severe hypoalbuminemia; in group B, 11 (64.7%) dogs had low albumin concentration, 4 of which showing severe hypoalbuminemia. The frequency of dogs with hypoalbuminemia or severe hypoalbuminemia was not significantly different between groups A and B.

Weeks 4-8

In group A at follow-up, BW increased in 16 (55.2%) dogs (**range,** from 0.5 to 4 kg), remained equal in 11 (37.9%) and decreased in 2 (6.9%); by arbitrarily considering BW as stable if increased or decreased by ≤2.5%, 14 (48.3%) of the 29 dogs had stable BW. The mean BW of dogs significantly increased by 6.2% (32 ± 15 kg; $P<0.01$), compared to baseline. BW was available for 10 out of 17 dogs of group B and was increased in 1 (10.0%) dog, equal in 5 (50.0%) and decreased in 4 (40.0%); BW was stable in 5 (50.0%) of the 10 dogs. The mean value did not differ from baseline (26 ± 12 kg; $P>0.05$) (Figure 1).

In group A, serum albumin concentration increased in 19 (65.5%) dogs, was equal in 2 (6.9%) and decreased in 8 (27.6%); by arbitrarily considering albumin as stable if increased or decreased by ≤5%, 8 (27.5%) of the 29 dogs had stable albumin. The mean albumin concentration significantly increased by 0.2 g/dL (2.6 ± 0.7 g/dL; $P<0.05$), compared to baseline. None of the 19 dogs with higher than baseline albumin had concentrations above the reference range. In group B, albumin concentration increased in 8 (47.1%) dogs, was equal in 2 (11.8%) and decreased in 7 (41.2%); albumin was stable in 4 (23.5%) of the 17 dogs. The mean value did not differ from baseline (2.4 ± 0.8 g/dL; $P>0.05$) (Figure 2).

Serum concentration of creatinine in group A increased in 6 (20.7%) dogs and decreased in the remaining 23 (79.3%); by considering creatinine as stable if increased or decreased by ≤20% (10), 13 (44.8%) of the 29 dogs had stable creatinine. In group B, creatinine concentration increased in 4 (23.5%) dogs, was equal in 2 (11.8%) and decreased in 11 (64.7%); creatinine was stable in 6 (35.3%) of the 17 dogs. In both groups, mean serum concentration of creatinine measured at 4-8 weeks did not statistically differ from baseline (group A: 2.0 ± 2.0 mg/dL; group B: 3.4 ± 3.3 mg/dL; $P>0.05$ for both).

In group A, serum concentration of urea increased in 9 (31.0%) dogs, was equal in 1 (3.4%) and decreased in 19 (65.5%); by arbitrarily considering urea as stable if increased or decreased by

≤20%, 13 (44.8%) of the 29 dogs had stable urea. The mean urea concentration did not statistically differ from baseline (53 ± 76 mg/dL; P>0.05). In group B, urea concentration increased in 5 (29.4%) dogs, was equal in 2 (11.8%) and decreased in 10 (58.8%); urea was stable in 6 (35.3%) of the 17 dogs. The mean urea concentration significantly decreased by 16 mg/dL (53 ± 35 mg/dL; P<0.05) (Figure 3).

In group A, the UPC ratio increased in 9 (31.0%) dogs and decreased in 20 (69.0%); by arbitrarily considering UPC ratio as stable if increased or decreased by ≤20%, 7 (24.1%) of the 29 dogs had stable UPC ratio. The mean UPC ratio did not differ from baseline (3.9 ± 4.9; P>0.05). In group B, the UPC ratio increased in 2 (11.8%) dogs, was equal in 1 (5.9%) and decreased in 14 (82.3%); UPC ratio was stable in 5 (29.4%) of the 17 dogs. The mean UPC ratio significantly decreased by 1.9 (2.4 ± 3.5; P<0.01) (Figure 4).

The exact time of examination performed within the 4-8 weeks interval did not differ between groups (5.5 ± 1.0 weeks, both groups).

Dogs with hypoalbuminemia

In group A, AA supplementation increased serum albumin concentration at 4-8 weeks compared to baseline in all 11 dogs with severe hypoalbuminemia (serum albumin <2.0 g/dL). The mean albumin concentration significantly increased by 0.70 g/dL (P<0.001). None of these dogs had detectable subcutaneous edema or ascites, based on physical examination or abdominal ultrasonography, respectively. No significant differences were observed for BW, serum creatinine and urea concentrations, or the UPC ratio. At 4-8 weeks, the 9 dogs with hypoalbuminemia between 2.0 and 2.7 g/dL had no significant change compared to baseline in BW, in serum albumin, creatinine and urea concentrations, or in UPC ratio.

In group B, the 4 dogs with severe hypoalbuminemia at 4-8 weeks compared to baseline had albumin concentration that was decreased in 2 of them and was equal and increased in one of each

253 remaining. Due to the limited number of cases (4 dogs), statistical analyses were not performed for
254 BW, serum albumin, creatinine and urea concentrations, or the UPC ratio. The 7 dogs with
255 hypoalbuminemia (serum albumin between 2.0 and 2.7 g/dL) had no significant change in BW,
256 serum albumin, creatinine and urea concentrations, or the UPC ratio at 4-8 weeks compared to
257 baseline.
258

259 **Discussion**

260 A significant increase in serum albumin concentration compared to baseline was evident in
261 proteinuric CKD dogs showing severe hypoalbuminemia (serum albumin <2.0 g/dL) when
262 receiving the AA supplementation. Along with a beneficial effect on serum albumin level,
263 supplementation with AA also increased dogs' BW, albeit mildly. The effect on BW was evident in
264 the whole group of proteinuric CKD dogs but not in those with severe hypoalbuminemia, possibly
265 due to the worse nitrogen balance of the latter cases. On the other hand, even though
266 supplementation with AA increased BW and serum albumin concentration in proteinuric CKD
267 dogs, it delayed the decrease of proteinuria and prevented lowering of urea.
268 Indeed, at follow-up, proteinuria and urea significantly decreased in dogs that did not receive AA
269 while they did not differ in dogs supplemented with AA. It is therefore possible that in these dogs
270 the reduced efficacy of the enalapril and commercial renal diet on either proteinuria or azotemia
271 was a direct consequence of the positive nitrogen balance and increased protein synthesis induced
272 by the AA supplementation. In fact, **diets lower in protein compared to maintenance diets** offer a
273 chance to reduce the overall renal trafficking of protein, and if serum protein can be lowered then
274 there is less risk of protein overload across the glomerular barrier, thus leading to less tubular
275 protein reabsorption and inflammation (3). The amount of proteins in the diet has a well-known
276 effect on the magnitude of proteinuria, and dogs fed with a **diet lower in protein compared to**
277 **maintenance diets** have reduced proteinuria, which can in turn improve serum albumin
278 concentration despite the reduction of albumin synthesis that can occur in CKD dogs (2,3,8). On the
279 other hand, because a too strict restriction of protein intake can lead to loss of BW and decreased
280 plasma albumin concentration, in proteinuric CKD dogs the protein amount administered daily with
281 food should be tailored to the degree of proteinuria; in these patients, dietary therapy should
282 minimize proteinuria and control plasma albumin concentration while not compromising the
283 nutritional status (8,24). The correct amount of proteins might differ depending on the dog's stage
284 of renal disease and entity of proteinuria (8). The commercial renal diets currently available in **dogs**

have lower protein compared to maintenance diets and it is possible they do not meet the minimum requirements in case of severe proteinuria (thus leading to hypoalbuminemia and loss of BW). Meanwhile, the degree of proteinuria is strictly associated with survival and CKD progression in dogs (1,3,11). In light of these findings, it is the authors' opinion that supplementation with AA should be carried out with caution in proteinuric CKD dogs, but it might be considered as an adjunctive therapy in severely hypoalbuminemic dogs in which the anti-proteinuric treatment has failed to control proteinuria and maintain plasma albumin concentration within normal limits.

This study has some limitations including its retrospective nature and consequent lack of blinding. It is, therefore, possible that some of the effects would have been different if cases were randomly allocated to receive or not the AA supplementation and the 2 groups were more homogeneous. Indeed, serum concentration of creatinine at baseline was significantly higher in dogs that did not receive the AA supplementation. Then, it cannot be excluded that administering AA supplementation to dogs with higher creatinine concentration is associated with detrimental effects on renal function. In addition, follow-up time of all dogs included in the study was short. A longer follow-up period might have allowed depicting additional differences between the groups. Additionally, even though owners were instructed to feed their dogs just with one of the two renal diets available, sometimes they switched to the other. However, the effect of this potential bias was probably minor because both commercial renal diets were expected to be **casually** provided to dogs. Furthermore, studies comparing the effect of different diets in dogs with CKD have not been published yet but it is likely that the two commercial renal diets used for the present study provided similar beneficial effects. The IRIS simply suggests the use of a renal diet, without offering specific guidance on a particular brand on the market (14).

Another limitation is represented by the fact that from medical records it was possible to retrieve BW but not the body or the muscle condition score of the dogs; the latter might have provided more information regarding the potential beneficial effect of AA supplementation . Furthermore, the increase of BW in dogs receiving supplementation of AA at follow-up might have been biased by

311 the concurrent presence of subcutaneous edema or abdominal effusion; however, none of the dogs
312 with severe hypoalbuminemia in the present group developed any of the above. With regard to the
313 same group of dogs, it is worth noting that at follow-up BW increased on average by only 6.2%,
314 thus the beneficial effect of AA supplementation would be questionable. However, by considering
315 the 16 dogs that had an increase of BW, the increase was from 0.5 to 4 kg, possibly suggesting a
316 more relevant gain.
317 Furthermore, the re-evaluation at 4-8 weeks may be considered a rather large interval, which might
318 have affected the results. Although this hypothesis is conceivable, the potential bias was evenly
319 distributed in the two groups, likely limiting the source of error.
320 Another factor that might have affected the study results is that the AA provided with the
321 supplementation were predominantly essential AA. Even though it has been demonstrated that
322 people with CKD have a decrease of circulating essential AA relative to non-essential AA, (25,26)
323 there are no data available regarding the AA **blood** profile of dogs with renal disease, particularly in
324 those affected by spontaneous proteinuric CKD. Determining the AA profile of these dogs might
325 prove useful to identify the specific AA that are needed to correct their imbalance.
326 Finally, even though BW and serum albumin concentrations have been historically considered as
327 insensitive and late indicators of malnutrition, in a previous study these values were considered
328 clinically useful in assessing the adequacy of the nutritional status in dogs with renal proteinuria (8).
329 Our results support the notion that serum albumin concentration represents a helpful indicator to
330 plan dietary modification in proteinuric dogs affected by spontaneous CKD.
331 In conclusion, proteinuric CKD dogs treated with enalapril and fed commercial renal diets that
332 received supplementation with AA had improved BW and serum albumin concentration, while
333 maintaining stable serum creatinine. However, administration of AA might delay the reduction of
334 proteinuria and prevent lowering of urea. In light of these findings, the authors propose to address
335 the AA supplementation to proteinuric dogs with severe hypoalbuminemia that are not adequately
336 controlled with standard treatments consisting of renal diets and ACEI. Relying on serum albumin

was useful to identify the benefits of dietary changes in proteinuric dogs with CKD. Further clinical trials are expected to be valuable in order to evaluate the impact of different AA formulations on BW, hypoalbuminemia, and survival time of dogs affected by severe proteinuric CKD.

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345 **References**

346 1) Jacob F, Polzin DJ, Osborne CA, Neaton JD, Kirk CA, Allen TA, Swanson LL. Evaluation
347 of the association between initial proteinuria and morbidity rate or death in dogs with
348 naturally occurring chronic renal failure. J Am Vet Med Assoc 2005;226:393-400.
349 2) Littman MP. Protein-losing nephropathy in small animals. Vet Clin Small Anim
350 2011;41:31-62.
351 3) Harley L, Langston C. Proteinuria in dogs and cats. Can Vet J 2012;53:631-638.
352 4) Nabity MB, Lees GE, Cianciolo R, Boggess MM, Steiner JM, Suchodolski JS. Urinary
353 biomarkers of renal disease in dogs with X-linked hereditary nephropathy. J Vet Intern Med
354 2012;26:282-293.
355 5) Zini E, Bonfanti U, Zatelli A. Diagnostic relevance of qualitative proteinuria evaluated by
356 use of sodium dodecyl sulfate-agarose gel electrophoresis and comparison with renal
357 histologic findings in dogs. Am J Vet Res 2004;65:964-971.
358 6) Grodecki KM, Gains MJ, Baumal R, Osmond DH, Cotter B, Valli VE, Jacobs RM.
359 Treatment of X-linked hereditary nephritis in Samoyed dogs with angiotensin converting
360 enzyme (ACE) inhibitor. J Comp Pathol 1997;117:209-225.
361 7) Matsuo S, Morita Y, Maruyama S, Manchang L, Yuzawa Y. Proteinuria and
362 tubulointerstitial injury: the causative factors for the progression of renal diseases. Contrib
363 Nephrol 2003;139:20-31.
364 8) Burkholder WJ, Lees GE, LeBlanc AK, Slater MR, Bauer JE, Kashtan CE, et al. Diet
365 Modulates Proteinuria in Heterozygous Female Dogs with X-Linked Hereditary
366 Nephropathy. J Vet Intern Med 2004;18:165-175.
367 9) Cortadellas O, Talavera J, Fernandez del Palacio MJ. Evaluation of the effects of a
368 therapeutic renal diet to control proteinuria in proteinuric non-azotemic dogs treated with
369 benazepril. J Vet Intern Med 2014;28(1):30-37.

10) Paltrinieri S, Solano-Gallego L, Fondati A, Lubas G, Gradoni L, Castagnaro M, et al. Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. J Am Vet Med Assoc 2010;236:1184-1191.

11) Lees GE, Brown SA, Elliott J, Grauer GE, Vaden SL. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). J Vet Intern Med 2005;19:377-385.

12) Jacob F, Polzin DJ, Osborne CA, et al. Clinical evaluation of dietary modification for treatment of spontaneous chronic renal failure in dogs. J Am Vet Med Assoc 2002;220:1163-1170.

13) IRIS Canine GN Study Group Standard Therapy Subgroup, Brown S, Elliott J, Francey T, Polzin D, Vaden S. Consensus recommendations for standard therapy of glomerular disease in dogs. J Vet Intern Med 2013;27 Suppl 1:S27-43.

14) International Renal Interest Society [homepage on the Internet] c2016 International Renal Interest Sosiety (IRIS) Available from: http://www.iris-kidney.com/guidelines/index.html Last accessed June 11, 2016.

15) Czekalski S, Hozejowski R. Intradialytic amono acids supplementation in hemodialysis patients with malnutrition: results of a multicenter cohort study. J Ren Nutr 2004;14:82-88.

16) Cano NJM, Fouque D, Leverve XM. Application of branched-chain amino acids in human pathological states: renal failure. J Nutr 2006;136:299S-307S.

17) Bellizzi V, Di Iorio BR, De Nicola L, Minutolo R, Zamboli P, Trucillo P, et al. Very low protein diet supplemented with ketoanalogs improves blood pressure control in chronic kidney disease. Kidney Int 2007;71:245-251.

18) Lawson JA, Lazarus R, Kelly JJ. Prevalence and prognostic significance of malnutrition in chronic renal insufficiency. J Ren Nutr 2001;11:16-22.

19) Feiten SF, Draibe SA, Watanabe R, Duenhas MR, Baxmann AC, Nerbass FB, Cuppari L. Short term effects of a very-low protein diet supplemented with ketoacids in nondialyzed chronic kidney disease patients. Eur J Clin Nutr 2005;59:129-136.

20) Klosterman ES, Moore GE, de Brito Galvao JF, DiBartola SP, Groman RP, Whittemore JC, et al. Comparison of signalment, clinicopathologic findings, histologic diagnosis, and prognosis in dogs with glomerular disease with or without nephrotic syndrome. J Vet Intern Med 2011;25:206-214.

21) Parker VJ, Freeman LM. Association between body condition and survival in dogs with acquired chronic kidney disease. J Vet Intern Med 2011;25:1306-1311.

22) Lulich JP, Osborne CA. Interpretation of urine protein-creatinine ratios in dogs with glomerular and nonglomerular disorders. Compend Cont Educ Pract Vet 1990;12:59-73.

23) Beatrice L, Nizi F, Callegari D, Paltrinieri S, Zini E, D'Ippolito P, Zatelli A. Comparison of urine protein-to-creatinine ratio in urine samples collected by cystocentesis versus free catch in dogs. J Am Vet Med Assoc 2010;236:1221-1224.

24) Parker VJ, Freeman LM. Focus on nutrition: nutritional management of protein-losing nephropathy in dogs. Compend Cont Educ Pract Vet 2012;34:E6.

25) Flugel-Link RM, Jones MR, Kopple JD. Red cell and plasma amino acid concentrations in renal failure. JPEN Journal of parenteral and enteral nutrition 1983;7:450-456.

26) Tizianello A, Deferrari G, Garibotto G, et al. Amino acid imbalance in patients with chronic renal failure. Contributions to nephrology 1989;75:185-193.

Figure legends

Fig. 1. Dot plot of BW in dogs in the group A and in the group B at baseline and after 4-8 weeks. After 4-8 weeks, BW significantly increased in the group A whereas did not change in the group B.

Fig. 2. Dot plot of serum albumin concentration in dogs in the group A and in the group B at baseline and after 4-8 weeks. After 4-8 weeks, albumin significantly increased in the group A whereas did not change in the group B.

Fig. 3. Dot plot of serum urea concentration in dogs in the group A and in the group B at baseline and after 4-8 weeks. After 4-8 weeks, urea significantly decreased in the group B whereas did not change in the group A.

Fig. 4. Dot plot of UPC ratio in dogs in the group A and in the group B at baseline and after 4-8 weeks. After 4-8 weeks, UPC ratio significantly decreased in the group B whereas did not change in the group A.

Tables

Table 1. Composition of the amino acid supplementation (100 grams).

Branched-chain aliphatic amino acids:	
isoleucine, leucine, valine	26 g
Aliphatic amino acids:	
threonine, arginine, lysine	24 g
Sulfur-containing amino acids:	
cysteine, methionine	7 g
Aromatic amino acids:	
tyrosine, phenylalanine	11 g
Heterocyclic amino acids:	
tryptophan, histidine	6 g
Carrier:	
glucose	10 g
sucrose	10 g
pregelatinized rice	6 g

436 Table 2. Age, BW, serum concentration of creatinine, urea and albumin, as well as UPC ratio in
437 dogs receiving AA supplementation (group A) and in dogs not receiving AA supplementation
438 (group B), at baseline.
439

	Group A (mean ± SD)	**Group B (mean ± SD)**
Age (years)	6 ± 3	6 ± 3
BW (kg)	30 ± 14	28 ± 15
Creatinine (mg/dL)	2.9 ± 1.3	4.6 ± 2.8
Urea (mg/dL)	66 ± 30	69 ± 45
Albumin (g/dL)	2.4 ± 0.7	2.4 ± 0.7
UPC ratio	4.1 ± 4.5	4.3 ± 4.6

440
441 SD, standard deviation; BW, body weight; UPC, urine protein to creatinine.
442