Effect of probiotic on hemato-biochemical alterations in dogs with chronic kidney disease

Kalyani Thakur, VM Dhoot, GR Bhojne, SV Upadhye and AP Somkuwar

Abstract
Kidney disease is more common in canine population today. Hence the need of medications for kidney diseases also increased recently. The purpose of the present study is to assess the efficacy of probiotics in the management of chronic kidney disease in dogs. A probiotic formulation of selected microbial strains administered through oral route in dogs with chronic kidney disease may extend renoprotection via intraintestinal extraction of toxic waste solutes. The aim behind the study is to prevent the production of major damaging uremic toxins by targeting the process of bacterial protein fermentation in the gut and improving renal function. Dogs with chronic kidney disease have been shown to experience a disturbed gut flora, which promotes the increased production of those harmful toxins. By supplementing with a specific combination of bacteria viz. *Streptococcus thermophilus, Lactobacillus acidophilus*, and *Bifidobacterium longum* (probiotics) and beneficial fibre i.e. psyllium (prebiotics), to support the growth of the good bacteria, it improves the health of the gut, suppressing the growth of harmful bacteria, thus decreasing the production of the toxins.

Keywords: chronic kidney disease, dogs and probiotics

Introduction
Kidney disease is a pathological process that affects any part of the kidney and may or may not be related with alterations in kidney function. It is characterized as a decrease in one or several kidney functions such as the loss of urine concentrating ability, followed by the elimination of small-molecular-weight molecules from the plasma, characterizing azotemia i.e. increase of Plasma-Urea and/or Plasma-Creatinine (Braun and Lefebvre, 2008) [5]. Kidney disease can occur abruptly or progressively over time. Chronic kidney disease is considered as an irreversible disease that is usually progressive (Polzin, 2013) [36]. Cases of azotemia are not always primary renal azotemia caused by parenchymal damage however can be prerenal or postrenal azotemia producing from reduced kidney perfusion and interferences with urine excretion, respectively (DiBartola, 2005) [9].

Regardless of the cause, it is mandatory to decrease the levels of blood urea nitrogen (BUN) and serum creatinine in CKD patients. Uremic illness occurs as a result of accumulation of organic waste products, so-called as Uremic Retention Solutes (URSs) that are normally excreted by the kidneys. A number of treatment modalities focusing URS have been proposed (Ramezani and Raj, 2014) [38]. Unfortunately, most of the treatment plans exhibit inherent disadvantages such as side-effects, high cost, etc.
The gut microflora is crucial for regulating the normal function of the intestinal barrier as it promotes immunological tolerance to antigens from nutrients or organisms, controls nutrient uptake and metabolism, and prevents propagation of pathogenic organisms (Power et al. 2014) [39]. Hence, the concept has emerged that dysregulation of intestinal microflora may have a significant role in kidney disease associated with dysbiotic gut microbiota (Vaziri et al. 2012) [52]. In recent times, the concept of 'probiotics' has been in limelight by health professionals (Alvarez-Olmos and Oberhelman, 2001; Doron and Gorbach, 2006) [1, 2]. Probiotics confer a benefit on the host after its administration (Huebner and Surawicz, 2006) [16]. Blood urea nitrogen and serum creatinine levels in pigs and rats were reported to decrease by probiotic bacteria (Ranganathan et al. 2005) [60]. Therefore, this study was designed to examine the effects of oral administration of probiotic bacteria in dogs with chronic kidney disease.

Materials and Methods
The present study was carried out at the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence and the cases referred to Teaching Veterinary Clinical Complex (TVCC)
Nagpur during the period from June 2019 to January 2020. In the present study, dogs of both the sexes and of different age groups, presented at TVCC, Nagpur Veterinary College, Nagpur and suspected for kidney diseases were subjected for estimation of blood urea nitrogen and serum creatinine as a component of routine clinical evaluation. In case of azotemic dogs, a detail history, physical examination findings, urinalysis and blood tests were carried out. Clinical data viz., age, sex, breeds, body weight and duration of illness were also recorded. The clinical observations included clinical signs and symptoms shown by the animals. The study included six dogs with kidney disease of different stages of chronic kidney disease. Dogs with elevated blood urea nitrogen and serum creatinine were clinically examined and projected for response to conventional fluid therapy for a period of 28 days. Depending upon the electrolyte imbalance and blood homeostasis, these cases were instituted intravenous fluids such as Ringers lactate, Dextrose with Normal Saline, or 5% Dextrose. The antibiotics were given based on urine AbST results. Dogs with hyperphosphatemia, Tab Sevelamer Hydrochloride 50 mg/kg b.w. and Sucralfate 1 gm was given per orally twice a day till the serum phosphorus levels returned to normalcy. The loop diuretic Furosemide was given @ 2 mg/kg b.w. twice a day in cases of oliguria. Inj. Erythropoietin 50 IU/kg, b.w. thrice a week was given in cases having severe anaemia (Hb. below 6.0 g/dL). Other symptomatic treatment such as Ondansetron (0.5 mg/kg b.w.) and Ranitidine (0.5 mg/kg b.w.) were given intramuscularly or orally in cases with vomiting and anorexia. All the affected dogs further received proprietary formulation of three strains of beneficial bacteria viz. Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacterium longum and a prebiotic (psyllium) orally in the form of capsule @ 3 capsules for dogs weighing ≥ 5 kg b.w. (2 in the morning and 1 in the evening) for a period of 28 days were given along with the conventional treatment. The dogs were monitored daily for improvement in clinical condition and the treatment regimen was modified as per the requirement. The owners counselling was done and they were advised to offer renal diet after ascertaining the hemato-biochemical parameters. Statistical analyses were conducted using WASP (version 2.0) software www.icargoa.res.in following the methods described by Snedecor and Cochran (2004) \[44\]. Comparisons were assessed using descriptive statistic, complementary randomised design and paired t-test. Variables with \(P<0.05\) were considered as statistically significant, variables with \(P<0.01\) were considered as statistically highly significant and variables with \(P>0.05\) were considered as statistically non-significant.

**Result and Discussion**

The affected dogs were classified into different stages of chronic kidney disease based on serum creatinine value which was done according to the methodology designed by International Renal Interest Society (IRIS) CKD Staging Guidelines (Modified 2019) that not only helped us with early diagnosis but also facilitated customize treatment and monitoring for individual dog (Table No. 1). The average age of dogs was 8.50 ± 1.32 years (range 5 to 13 years) (Table No. 1). These findings were in accordance with Karunanithy et al. (2019) \[19\] and Thade et al. (2019) \[27\], Bhojne et al. (2016) \[10\] recorded highest prevalence of renal insufficiency in 6 to 9 years of age group followed by 10 to 13 years of age group and 14 and above age group. With ageing, renal function decreases as a result of renal vascular insufficiency in 6 to 9 years of age group followed by 10 to 13 years of age group and 14 and above age group. An interesting fact about progression of kidney disease is gender disparity where a male dog shows a faster rate of progression of certain CKD because of the effects of the interaction of circulating steroids mainly androgens on specific kidney receptors which exaggerate kidney diseases, whereas estrogens confer renal protection (Lu et al. 2007).

**Table 1: Signalment of the patients**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Patient name</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Body weight (kg)</th>
<th>Breed</th>
<th>Sr. Creatinine (mg/dl)</th>
<th>UPC</th>
<th>IRIS classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leo</td>
<td>6.5</td>
<td>Male</td>
<td>48.0</td>
<td>St. Bernard</td>
<td>4.3</td>
<td>2.27</td>
<td>Stage 3</td>
</tr>
<tr>
<td>2</td>
<td>Pari</td>
<td>9.0</td>
<td>Female</td>
<td>25.0</td>
<td>Labrador</td>
<td>3.6</td>
<td>1.74</td>
<td>Stage 3</td>
</tr>
<tr>
<td>3</td>
<td>Tony</td>
<td>11.5</td>
<td>Male</td>
<td>14.0</td>
<td>Non-Descript</td>
<td>5.3</td>
<td>3.81</td>
<td>Stage 4</td>
</tr>
<tr>
<td>4</td>
<td>Daisy</td>
<td>6.0</td>
<td>Female</td>
<td>33.5</td>
<td>Rottweiler</td>
<td>4.9</td>
<td>2.88</td>
<td>Stage 3</td>
</tr>
<tr>
<td>5</td>
<td>Lola</td>
<td>13.0</td>
<td>Female</td>
<td>20.0</td>
<td>Dalmatian</td>
<td>6.8</td>
<td>6.84</td>
<td>Stage 4</td>
</tr>
<tr>
<td>6</td>
<td>Danny</td>
<td>5.0</td>
<td>Male</td>
<td>31.0</td>
<td>Labrador</td>
<td>5.2</td>
<td>1.51</td>
<td>Stage 4</td>
</tr>
<tr>
<td>Average ± SE</td>
<td>8.50 ± 1.32</td>
<td>28.58 ± 4.85</td>
<td></td>
<td>5.01 ± 0.44</td>
<td>3.17 ± 0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The breeds reported for the present study were Labrador retriever, Rottweiler, St. Bernard, Dalmatian, and Non-Descript. The present findings were in agreement with Tufani et al. (2015) \[49\] and Nabi et al. (2018) \[10\] who recorded highest prevalence of renal failure in Labrador followed by German shepherd and Pomeranian. Labrador retriever is the most common breed diagnosed with bladder tumors (Krawiec, 1989) \[22\]. There was not enough population size in the present study in order to estimate the prevalence of breed on chronic kidney disease.

**Table 2: Chief complaint and clinical signs of the patients**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Patient name</th>
<th>Patient complaint and Clinical signs</th>
<th>Duration of illness (Days)</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leo</td>
<td>Inappetence, Vomiting, Diarrhoea, Dullness, Anaemia</td>
<td>15</td>
<td>Commercial</td>
</tr>
<tr>
<td>2</td>
<td>Pari</td>
<td>Anorexia, Vomiting, Lethargic, Polyuria</td>
<td>10</td>
<td>Homemade + Commercial</td>
</tr>
<tr>
<td>3</td>
<td>Tony</td>
<td>Anorexia, Vomiting, Diarrhoea, Anaemic, Dental Tartar and</td>
<td>21</td>
<td>Homemade</td>
</tr>
</tbody>
</table>
The data regarding the chief clinical signs exhibited by each patient, duration of illness and diet history are summarized in Table No. 2. It was observed that inappetence/anorexia and vomiting were the cardinal clinical signs observed in 100% of the cases. Anaemia related to chronic kidney disease was noticed followed by diarrhoea and oral ulceration with dental tartar and halitosis. Majority of the patients exhibited dull and lethargic behaviour at presentation. Polypuria was seen in only two dogs. The observations of various clinical signs in the present study were in accordance with Tripathi and Mehta (2010) [13] and Bartges (2012) [2] who also reported the similar clinical findings in the dogs affected with chronic kidney diseases. Oburai et al. (2015) [32] and Duneaevich et al. (2020) [13] also observed indistinguishable symptoms in dogs suffering from chronic renal failure viz. anaemia, followed by vomiting, dullness and weight loss, oral ulcer, hypertension, polyuria and polydipsia, recumbency and blindness.

The hematological investigation of packed cell volume (PCV), hemoglobin and total erythrocyte count (TEC) value in all the affected dogs were slightly lower compared to referral limit and did not reveal any significant differences during various days of treatment protocol (Table No. 3). These observations were in line with Sawale et al. (2012) [43], Oburai et al. (2015) [32] and Devipriya et al. (2018) [8] who also noticed remarkable reduction in the haemoglobin, PCV and total erythrocyte count on day first of study. Duneaevich et al. (2020) [13] documented lower haemocrit (<37.1%) and TEC (4.0 x 10⁹/L) concentration in dogs suffering from acute on chronic kidney disease. The root cause for the decrease in these counts could be the impaired ability of the kidneys to produce a sufficient quantity of erythropoietin and shortened survival period of red blood cells. Other secondary causes for anaemia could be iron-deficiency anaemia or Vitamin B₁₂/folate deficiency, poor nutrition, and spontaneous blood loss.

### Table 3: Average ± S.E. of haematological parameters of dogs during treatment period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Referral range</th>
<th>0th Day</th>
<th>7th Day</th>
<th>14th Day</th>
<th>21st Day</th>
<th>28th Day</th>
<th>CD (0.01)</th>
<th>CD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume</td>
<td>37 - 55%</td>
<td>35.35 ± 4.98</td>
<td>34.21 ± 4.67</td>
<td>35.73 ± 4.42</td>
<td>36.78 ± 3.98</td>
<td>35.88 ± 3.62</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>12 - 18 gm%</td>
<td>10.63 ± 0.82</td>
<td>10.16 ± 0.67</td>
<td>10.38 ± 0.71</td>
<td>10.73 ± 0.61</td>
<td>10.73 ± 0.51</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>5.5 - 8.5 10⁶/µL</td>
<td>4.76 ± 0.39</td>
<td>4.37 ± 0.44</td>
<td>4.52 ± 0.39</td>
<td>4.63 ± 0.34</td>
<td>4.69 ± 0.26</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TLC</td>
<td>18 - 17 x 10³/µL</td>
<td>18.3 ± 1.08a</td>
<td>16.13 ± 1.26a</td>
<td>14.58 ± 1.32ab</td>
<td>13.13 ± 1.84 b</td>
<td>12.33 ± 1.54 b</td>
<td>4.184</td>
<td></td>
</tr>
</tbody>
</table>

Different superscripts row-wise indicates significance, NS indicates non-significant difference, CD = Critical difference

There was a significant difference (P<0.05) in the average TLC (10³/µL) values between the 0th day and 28th of treatment at statistical level (Table No. 4). Sumit et al. (2018) [46] found higher mean values of total leucocyte count (16.40 ± 2.73 x 10⁹/mm³) in all affected dogs when compared to the referral value. Likewise, Karunanithy et al. (2019) [39] and Duneaevich et al. (2020) [13] also recorded increased levels of total leucocyte count in dogs affected with chronic kidney diseases. The possible cause of leukocytosis in the present study could be the increased production of TLC by the bone marrow in response to an infection or inflammation and stress in any part of the kidneys. There was a significant (P<0.05) decrease in the TLC values and were found in the normal range at the end of treatment period. This notable reduction in the TLC values after treatment could be attributed to the resolution of infection or inflammation.

The average concentration of BUN (mg/dl) in all dogs before treatment was 67.85 ± 5.94, and post treatment were 56.25 ± 7.10, 51.86 ± 7.54, 42.13 ± 6.61 and 30.51 ± 4.16 on 7th, 14th, 21st, and 28th day. A highly significant decrease (P<0.01) in the blood urea nitrogen value was observed on 21st day and 28th day post treatment compared to 0th day (Table No. 4).

There was a significant difference (P<0.05) in the average BUN (mg/dl) values between 0th day and 28th of treatment at statistical level (Table No. 4). Sumit et al. (2018) [46] found higher mean values of total leucocyte count (16.40 ± 2.73 x 10⁹/mm³) in all affected dogs when compared to the referral value. Likewise, Karunanithy et al. (2019) [39] and Duneaevich et al. (2020) [13] also recorded increased levels of total leucocyte count in dogs affected with chronic kidney diseases. The possible cause of leukocytosis in the present study could be the increased production of TLC by the bone marrow in response to an infection or inflammation and stress in any part of the kidneys. There was a significant (P<0.05) decrease in the TLC values and were found in the normal range at the end of treatment period. This notable reduction in the TLC values after treatment could be attributed to the resolution of infection or inflammation.

The increased concentration blood urea nitrogen observed in the present study could be attributed to various prerenal causes like cardiac decompensation, water depletion due to decreased intake or excessive loss, increased protein catabolism, and high protein diet whereas renal causes includes acute glomerulonephritis, chronic nephritis, polycystic kidney disease, nephrosclerosis, and tubular necrosis and postrenal causes comprises of all types of obstruction of the urinary tract, such as stones, enlarged prostate gland, tumors. There could be few extrarenal causes also responsible for increased BUN such as protein intake, gastrointestinal bleeding, catabolic states, malnutrition, heart failure, dehydration, use of glucocorticoids, and hepatic urea synthesis. An elevated concentration of blood urea nitrogen was observed before treatment which gradually decreased after the treatment course and these findings were in correlation with Palmquist (2006) [33], Ross (2006) [44], Sumit et al. (2018) [46], Karunanithy et al. (2019) [39], Nakang et al. (2019) [33] and Duneaevich et al. (2020) [13]. All the authors have reported an increased blood urea nitrogen level in dogs suffering from kidney diseases.

### Table 4: Average ± S.E. of biochemical parameters of dogs during treatment period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Referral range</th>
<th>0th Day</th>
<th>7th Day</th>
<th>14th Day</th>
<th>21st Day</th>
<th>28th Day</th>
<th>CD (0.01)</th>
<th>CD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea nitrogen</td>
<td>12 - 25 mg/dl</td>
<td>67.85a ± 6.94</td>
<td>56.25ab ± 7.10</td>
<td>51.86ab ± 7.54</td>
<td>42.13bc ± 6.61</td>
<td>30.51c ± 4.16</td>
<td>25.948</td>
<td>19.180</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.5 - 1.5 mg/dl</td>
<td>5.01a ± 0.44</td>
<td>4.53ab ± 0.49</td>
<td>4.03abc ± 0.49</td>
<td>3.40bc ± 0.44</td>
<td>2.91bc ± 0.48</td>
<td>1.383</td>
<td></td>
</tr>
<tr>
<td>Serum phosphorus</td>
<td>2.2 - 5.5 mg/dl</td>
<td>7.53a ± 0.58</td>
<td>6.21ab ± 0.55</td>
<td>5.93b ± 0.48</td>
<td>5.63b ± 0.45</td>
<td>5.46b ± 0.36</td>
<td>NS</td>
<td>1.438</td>
</tr>
<tr>
<td>Serum sodium</td>
<td>141 - 153 mEq/L</td>
<td>135.00a ± 2.46</td>
<td>140.33ab ± 2.36</td>
<td>142.66bc ± 1.99</td>
<td>146.95cd ± 1.35</td>
<td>148.96cd ± 1.16</td>
<td>7.661</td>
<td>5.662</td>
</tr>
<tr>
<td>Serum potassium</td>
<td>3.7 - 5.8 mEq/L</td>
<td>3.96a ± 0.24</td>
<td>4.60ab ± 0.34</td>
<td>4.96ab ± 0.24</td>
<td>5.11bc ± 0.21</td>
<td>5.36c ± 0.16</td>
<td>0.991</td>
<td>0.733</td>
</tr>
<tr>
<td>Serum chloride</td>
<td>105 - 115 mEq/L</td>
<td>103.33a ± 1.26</td>
<td>106.11ab ± 1.33</td>
<td>104.95abc ± 2.00</td>
<td>108.96bc ± 2.08</td>
<td>110.86c ± 1.83</td>
<td>NS</td>
<td>5.068</td>
</tr>
</tbody>
</table>
The highly significant decrease in the BUN concentration in the present study could be an effect of probiotic therapy used in all dogs that has a property of catabolizing uremic solutes in the gut. Palmquist (2006) [33] observed a very clear relationship between use of the probiotic and decreasing azotemia where 100% of cases showed decrease in BUN value varying from 4.7% to 36.5 per cent. Ranganathan et al. (2009) [19] also observed a mean change in BUN concentration of -2.93 mmol/L during probiotic treatment period differing significantly from the mean change in BUN concentration of 4.52 mmol/L during the placebo period by feeding an oral probiotic selected bacteria in stage 3 & 4 chronic kidney disease. Koppe et al. (2015) [21] explained that administration of probiotics during chronic kidney diseases helps in removing uremic retention solutes (URSs). Thus, when the production of URSs primarily by protein degradation is not completely blocked by a low-protein diet, modelling intestinal microbiota could be considered as an additional beneficial intervention by reducing the conversion of amino acids into trimethylamine n-oxide, p-cresyl sulphate, or indoxyl-sulfate.

An increased concentration of serum creatinine was observed before treatment which gradually decreased after the treatment course and these findings were in accordance with Ross (2006) [41], Sawale et al. (2012) [43], Obura et al. (2015) [32], Sumit et al. (2018) [40], Karunanithy et al. (2019) [19], Nakang et al. (2019) [31] and Dunaevich et al. (2020) [13]. Each author has reported an increased serum creatinine level in dogs suffering from kidney diseases. The values of the serum creatinine concentration were significantly decrease before and after treatment in the present study could be due to the probiotic supplementation given in all cases. Palmquist (2006) [33] observed a very clear relationship between use of the probiotic and decreasing azotemia where 85.7% (6/7) of cases showed decrease in creatinine value ranging from 10% to 51.9 per cent. McCain et al. (2011) [27] also reported that the serum urea nitrogen and creatinine concentrations decreased after 60 days of probiotic administration in azotaemic big cats. Jo et al. (2014) [17] also documented significant decrease in the blood urea nitrogen and serum creatinine concentration on 30th day of dietary probiotics treatment along with supportive conventional treatment in a dog diagnosed with chronic kidney disease.

There was marked reduction in the values of serum phosphorus between day 0th, 7th, 14th, 21st and 28th day of treatment. A significant decrease (P<0.05) in serum phosphorus value was observed on 0th day, 14th day and 28th day of post treatment. The possible reason for such increased values of serum creatinine in the present study could be attributed to increase production of creatinine by the muscles especially in large breed of dogs with large muscle mass, in the study such as St. Bernard, Labrador retriever and Rottweiler. Another reason could be reduction in extracellular fluid volume due to dehydration or intestinal absorption of exogenous creatinine (Lefebvre et al. 2015) [6].

Urea is hydrolysed by the urease-producing probiotic species and maintains a concentration gradient that supports diffusion of urea from the blood to the gastrointestinal tract lumen. Thus, reduces uremia, serum creatinine concentration and number of fatalities (Wynn, 2009) [54]. The average concentration of serum creatinine (mg/dl) in all dogs before treatment was 5.01 ± 0.44 and post treatment were 4.53 ± 0.49, 4.03 ± 0.49, 3.40 ± 0.44 and 2.91 ± 0.48 on 7th, 14th, 21st, and 28th day. There was significant difference between day 0th, 7th, 14th, 21st and 28th day of treatment. A significant decrease (P<0.05) in the serum creatinine value was observed on 0th day, 14th day and 28th day of post treatment. There was marked reduction in the values of serum creatinine at different intervals of treatment (n = 6/6)

<table>
<thead>
<tr>
<th>Total protein</th>
<th>5.4 - 7.7 gm/dl</th>
<th>6.34 ± 0.33</th>
<th>5.79 ± 0.25</th>
<th>5.92 ± 0.38</th>
<th>5.76 ± 0.28</th>
<th>5.67 ± 0.25</th>
<th>NS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>2.3 - 3.8 gm/dl</td>
<td>2.42 ± 0.17</td>
<td>2.54 ± 0.14</td>
<td>2.74 ± 0.20</td>
<td>3.09 ± 0.28</td>
<td>3.17 ± 0.32</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Different superscripts row-wise indicates significance, NS indicates non-significant difference, CD = Critical difference

![Blood Urea Nitrogen vs Serum Creatinine at different intervals of days](image)

**Fig 1:** Relationship between blood urea nitrogen and serum creatinine at different intervals of treatment (n = 6/6)
The changes in the average electrolyte values of serum sodium, serum potassium and serum chloride (mEq/L) before and after treatment are mentioned in Table No. 4. The difference of serum sodium and serum potassium were highly significant \((P<0.01)\) in all the affected dogs before and after treatment, similarly the value of serum chloride also showed significant difference \((P<0.05)\) between different days of treatment. Results of the present study were in line with Martínez and Carvalho et al. (2010) [26], Meuten (2012) [28] and Sumit et al. (2018) [46] delineated hyponatremia with slight reduction in the serum sodium value in chronic kidney disease affected dogs. Slight hyponatremia was observed in the present study and the prominent reason could be volume depletion associated with extrarenal losses such as dehydration, vomiting and diarrhoea observed in majority of the affected dogs or renal losses such as decreased glomerular filtration rate in advanced stages of CKD or diuretic therapy induced by private practitioner in the patients.

The results of the present study showed slight depletion of serum potassium levels on 0\(^{\text{th}}\) day which is in accordance with DiBartola (2006) [10], Stockham and Scott (2008) [45], and Langston (2017) [23] who also opined that hypokalemia is more likely to occur in patients of chronic kidney disease and hyperkalemia occurs only until the glomerular filtration rate falls below 15-20 mL/min. Mild hypokalemia observed in the present study could be attributed to various reasons such as prolonged anorexia, loss of muscle mass and excessive renal wasting associated with polyuria in the affected dogs especially in the later stages of chronic kidney disease. Gastrointestinal loss of potassium such as vomiting and diarrhea also contributed to hypokalemia in the affected dogs. The observation of the present study with regard to serum chloride concentration was in agreement with Meuten (2012) [28], Sumit et al. (2018) [46] and Dunaevich et al. (2020) [13] who also reported significant lower concentration of serum chloride in dogs suffering from chronic kidney disease. Hypochloremia generally occurs in conjugation with hyponatremia (Stockham and Scott, 2008) [45] and this concept also implies in the present study wherein hyponatremia was observed on 0\(^{\text{th}}\) day that gradually increased with the help of conventional fluid therapy, simultaneously the chloride value increased with the effect of treatment and the changes were statistically significant.

The biochemical investigation did not reveal any significant differences in serum total protein and serum albumin among all the affected dogs during different days of treatment. The result of the present study regarding total protein concentration was in accordance with Sumit et al. (2018) [46] and Dunaevich et al. (2020) [13] who also observed non-significant changes in the serum total protein concentration in dogs suffering from chronic kidney disease. Hypoproteinaemia is common in dogs with chronic kidney disease and the primary reason could be proteinuria which results due to malfunctioning of normal renal handling of proteins. Additionally, diets with very high amount of protein intake also have deleterious effects on kidneys of dogs. Completely restricting or excessively limiting the dietary protein intake can further aggravate protein malnutrition and thus, high quality protein sources were advised in the diet formulation in order to minimize the risk of hypoproteinaemia in dogs.

### Table 5: Average ± S.E. of urinalysis of dogs during treatment period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Referral range</th>
<th>0th Day</th>
<th>14th Day</th>
<th>28th Day</th>
<th>CD (0.01)</th>
<th>CD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine specific gravity</td>
<td>1.001-1.065</td>
<td>1.012a± ± 0.002</td>
<td>1.020ab ± 0.003</td>
<td>1.023b ± 0.002</td>
<td>NS</td>
<td>0.008</td>
</tr>
<tr>
<td>UPC ratio</td>
<td>&lt;0.2</td>
<td>3.17a± ± 0.80</td>
<td>1.44b± ± 0.39</td>
<td>0.75b± ± 0.18</td>
<td>NS</td>
<td>1.597</td>
</tr>
</tbody>
</table>

Different observed superscripts row-wise indicates significance. NS indicates non-significant difference, CD = Critical difference

The observed values of urine specific gravity in all dogs were found significantly decreased on 0\(^{\text{th}}\) day of study and the values showed improvement at the end of the therapy. There was a statistical significant difference \((P<0.05)\) observed between 0\(^{\text{th}}\) day and 28\(^{\text{th}}\) day of treatment course (Table No. 5). The observation with respect to urine specific gravity in the present study commensurate with Forterre et al. (2004) [14], Oburai et al. (2015) [32], Karumanthy et al. (2019) [19] and Dunaevich et al. (2020) [13]. These authors have also reported significant decrease in urine specific gravity of dogs with chronic kidney disease when compared with normal healthy dogs. The urine specific gravity ranging between 1.008-1.020 is considered as isosthenuria where the urine specific gravity is similar to serum osmolality. Braun and Levebvre (2008) [5] opined isosthenuria as a frequent finding in dogs with chronic renal failure. Braun and Levebvre (2015) [6] states that a urine specific gravity < 1.008 or > 1.030 reflects the diluting or concentrating function of kidneys that certainly preclude chronic renal failure in dogs. This might be the potential reason of isosthenuria observed in the present study.

The average urine protein to creatinine ratio (UPC) in all dogs before treatment was 3.17 ± 0.80 and the post treatment values for 14\(^{\text{th}}\) and 28\(^{\text{th}}\) day were 1.44 ± 0.39 and 0.75 ± 0.18, respectively. The average value was remarkably increased on the 0\(^{\text{th}}\) day of treatment; however a significant decrease \((P<0.05)\) in the urine protein to creatinine ratio was observed between 0\(^{\text{th}}\) day and 14\(^{\text{th}}\) day of therapy and further decline on 28\(^{\text{th}}\) day of treatment. The result of urine protein to creatinine ratio (UPC) of the present study correlated with Forterre et al. (2004) [14] who documented UPC of 1.62 in dogs with renal disease, Pillai et al. (2012) [33] who reported UPC of >3.5 mg/mmol in a dog with nephrotic syndrome and Oburai et al. (2015) [32] who also noticed significantly increased UPC of 2.29 ± 0.25 in dogs with chronic renal failure. Nakang et al. (2019) [31] observed a significantly high UPC ratio of 2.25 ± 0.16 in dogs with renal dysfunction compared to healthy dogs with 0.03 ± 0.01 UPC ratio. In normal dogs, the urine protein to creatinine ratio (UPC) of <0.2 is considered as non-proteinuric however, in dogs with chronic kidney disease, the urine protein to creatinine ratio of >1.0 is associated with threefold greater risk of developing uremic crisis and death (Vaden and Elliott, 2016) [51]. Harley and Langston (2012) [15] quoted urine protein to creatinine ratio of > 2.0 as severe proteinuria which is a significant clinicopathological sign of glomerular disease and very high proteinuria with UPC >10.0 is suggestive of acquired or reactive glomerular amyloidosis. According to Nabyti (2011) [29], for UPC monitoring in dogs, if the UPC value has changed from the baseline i.e. between 0.5 and 12, it must either increase or decrease by a minimum of 35-80% with a higher per cent change required at lower UPC values that correlates with the results of present study.

Out of the total number of dogs, patient ID No. 02, 04 and 06 showed remarkable recovery in terms of resolution of clinical symptoms and significant improvement in the blood and urine...
parameters. All these dogs were advised to maintain on probiotic supplements even after the treatment course period along with other oral conventional supplementation. Recurrences of clinical symptoms were not observed during their follow-up visits until 1 to 6 months. Patient ID No. 01 and 03 were also advised to maintain on probiotic supplements; however, the owners could not afford the expenses of capsules and discontinued the treatment and were maintained on other oral conventional supplementation. Recurrences of symptoms were noticed after 3-4 months of their follow-up visit. Patient ID No. 05 also successfully completed the study period with partial correction of clinical signs such as improvement in appetite, fecal consistency and anemia. The blood and urine parameters were also significantly improved however, the owner refused to continue with the treatment plan further looking after the expenses and costs of medicines. The dog succumbed almost after 40 days of post treatment.

References
26. Martinez PP, Carvalho MB. Participation in the Renal Excretion of Calcium, Phosphorus, Sodium and Potassium Homeostasis in Healthy Dogs and Dogs with Chronic Kidney Disease. Pesquisa Veterinária Brasileira


