IMPACT OF DIETARY SUPPLEMENTATION OF COATED SODIUM BUTYRATE AND/OR POSTBIOTIC ON GROWTH PERFORMANCE, HEALTH STATUS AND OXIDATIVE BIOMARKERS IN GERMAN SHEPHERD DOGS

By

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ABSTRACT

Canine gut microbiota is crucial in metabolism, immune tolerance and nutrients absorption. The present study aims to determine the impact of dietary supplementation of coated sodium butyrate and/or postbiotics on health performance, antioxidant activity, cholesterol level and calcium level in GSD. In the study, twelve male GSD (Age of six months) were randomly allocated into four dietary treatments as follows: 1) Control group was fed only the basal diet without any food additive; 2) BUTR group was fed the basal diet supplemented with coated sodium butyrate; 3) POST group was fed the basal diet supplemented with postbiotics; 4) MIX group was fed on the basal diet supplemented with mix of postbiotics and coated sodium butyrate. Results of the trial reported that, the average daily gain improved in the MIX group followed by BUTR and POST groups while the lowest gain was recorded in the control group (P=0.02). Body condition scoring (BCS) was enhanced by the food additives used (P=0.02). Blood serum indices showed improving significantly serum calcium level (P=0.02) and lowering total cholesterol level in the MIX and BUTR groups respectively. Malondialdehyde (MDA) tended to decrease in MIX group followed BUTR and POST groups. Fecal scoring was the best in the MIX group followed by BUTR one. The fecal moisture was significantly decreased in all treatment groups compared to the control one (P=0.01). In conclusion, coated sodium butyrate and/or postbiotic could be used safely in GSD food to improve gut health reflected on healthy growth and general health condition of dogs.

Keywords:

German shepherd dogs, coated butyrate, postbiotic, growth performance, antioxidant biomarkers.
INTRODUCTION

Feeding good nutritious diets to dogs and cats are becoming an increasingly vital part of ethical pet keeping. Pet owners now want their pets to live a long and healthy life. Appropriate diet and nutraceutical supplements appear to improve quality of life, as evaluated by reduced disease incidence and the ability to maintain the dog general health (Bontempo, 2005). Several studies have found that the gut microbiota has an important role in the of the host health. The gut bacteria have been shown to influence the host's nutrient intake, energy expenditure, physiological, and metabolic activities, as well as drive the immune response, adding to the host's overall health (Havenaar, 2011). The gut microbiota in dogs and cats performs a variety of functions that, the animal body would not be able to do at its best without it. In dogs and cats, the large intestine has the highest microbial colonization and the most intensive microbial activity (Schafer-Evans, 2019). Thus an imbalance of gut microbiota generated by diet, use of antibiotics, or infections, can be detrimental for host equilibrium (Scarpellini et al., 2021).

There are a variety of nutritional strategies for gut health improvement such as using probiotics, prebiotics, synbiotics, exogenous food enzymes, essential oils and herbs, organic acids and/or their salts and many other food additives for dogs. However, emerging evidence shows how the use of living organisms, namely probiotics, is not devoid of virulence emergence and antibiotic resistance development over time (Daniali et al., 2020). Thus, there is a need for use of safer and equally effective gut microbiota modulatory agents. One very promising chance is represented by bacterial products and the components of probiotics, namely “postbiotics” (Żółkiewicz et al., 2020a). Postbiotics are probiotic bacteria metabolites that have a probiotic effect despite the absence of living cells (Tsilingiri and Rescigno, 2013). Immunomodulatory, anti-inflammatory, antioxidant, and anti-cancer activities are all shown by postbiotics (Żółkiewicz et al., 2020b). Butyric acid is generated in the intestinal lumens of monogastric animals by bacterial fermentation of unabsorbed carbohydrates (Tan et al., 2014; Vinolo et al., 2011). Butyrate has a wide range of biological impacts including supplying energy to intestinal epithelial cells, salt and water absorption, affect epithelial cell proliferation and differentiation, villi development, and gut defense systems. Butyrate improves barrier function, has antibacterial activity, and influences the immune system positively (Donohoe et al., 2011; Kumari et al., 2013). Butyrate has been shown to have great potential in enhancing health and performance of many animal species (Bolívar Ramírez et al., 2017; Gümüş et al., 2020; Liu et al., 2017; Wu et al., 2018; Yu et al., 2017; Zhang et al., 2011; Zou et al., 2019).
Scarce data is available regarding the effect of dietary supplementation of coated (Slowly released butyrate) products and/or postbiotics in sensitive dog breeds in Egypt. Based on such concept, the goal of the present study was to investigate the effects of slowly released coated sodium butyrate product (CM3000®) and/or Postbiotic (Lactéol fort®) on general performance and health of sensitive German shepherd dogs under Egyptian conditions.

**Material and methods:**

**Ethical approval.**

Study was approved by the Institutional Animal Care and Use Committee (IACUC), Cairo University, Egypt (Vet CU12/10/2021/387).

**Food Additives Used:**

1-CM3000® is a commercial 30% microencapsulated sodium butyrate. CM3000® keeps releasing slowly and continuously in both small and large intestine. CM3000® is manufactured by Hangzhou King Techina Feed Co., Ltd, China.

2-Lactéol fort® is a commercial post-biotic capsules. Each capsule contains lyophilized inactive microbial bodies corresponding to Lactobacillus delbruekii and Lactobacillus fermentum 5 billions. Lactéol fort® is manufactured Rameda by Tenth of Ramadan for Pharmaceutical Industries and Diagnostic Reagents Co., Egypt.

**Experimental design:**

The feeding trial was carried out at a private dog farm located in Cairo, Egypt and lasted for 45 days. Twelve healthy dogs, male German shepherd breed, 6 months old of nearly the same weight (12-13 Kg), was randomly assigned to four dietary treatment groups (n= 3/group). Blood and serum health parameters of all dogs were within normal physiological range before the onset of the feeding trial. All dogs were vaccinated with Vanguard® vaccine and dewormed using Drontal Plus® before the onset of the experiment. Each dog in each group was housed in individual kennel and had access to an outside garden for exercise and socialization with other dogs for 1 h daily. Fresh water was available ad libitum throughout the experiment. Kennels were cleaned twice daily. Dogs were daily checked by the same veterinarian during the whole study period. The trial included 7-days adaptation period followed by 45-days of data collection period. Dogs received a commercial, nutritionally complete, extruded dry dog food (Jessie®, Belgium). Basal diet was analyzed before the onset of the experiment according to procedure of Association of Official Analytical Chemists (AOAC, 2000). (Table 1) illustrated the chemical composition of the basal diet.
Table (1): Chemical composition of the basal diet.

<table>
<thead>
<tr>
<th>Item</th>
<th>% (DM basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>9</td>
</tr>
<tr>
<td>DM%</td>
<td>91</td>
</tr>
<tr>
<td>CP%</td>
<td>28</td>
</tr>
<tr>
<td>EE%</td>
<td>14</td>
</tr>
<tr>
<td>CF%</td>
<td>3.5</td>
</tr>
<tr>
<td>Ash%</td>
<td>8.5</td>
</tr>
<tr>
<td>NFE%</td>
<td>37</td>
</tr>
</tbody>
</table>

*ME=Metabolizable energy.=346.5Kcal/100g.

DM=Dry matter, CP=Crude protein, EE=Ether extract, CF=Crude fiber, NFE=Nitrogen free extract.

Each dog in all different treatment groups was fed individually and the amount of food provided daily was equal according to the recommendation of Association of American Feed Control Officials (AAFCO, 2000).

The first group (CON) was fed on basal un-supplemented diet. The second group (BUTR) dogs was fed on the basal diet supplemented with coated butyrate (CM3000®) 0.5 g/dog/day. The third group (POSTB) was fed on the basal diet supplemented with postbiotic capsules (Lactéol fort®) the postbiotic was added to the diet at a dose 1 capsule/dog/day. The fourth group (MIX) dogs was fed on the basal diet supplemented with the postbiotic and the coated butyrate with the same doses each. Food was offered twice daily.

Measurements and Sampling:

1) Growth performance parameters.

Body weight, body weight gain, and body condition score (BCS) were recorded weekly. The nine scale scoring system was applied (Bruni et al., 2020).

2) Blood Serum indices and antioxidant biomarkers.

Blood samples were collected at the end of the experimental period from cephalic vein from all dogs and serum was separated according to (Mundim et al., 2007). Serum was stored in -20°C till the time of analysis. All analytical procedures of the blood and serum samples were carried out at Hormones Analysis and Immunity Measurement lab; Agricultural Research Center, Giza, Egypt. Samples were analyzed for complete blood count (CBC), serum total cholesterol, calcium and malondialdehyde (MDA) as an antioxidant biomarker. Serum samples were analyzed using commercial kits (Biodiagnostic-Dokki - Giza- Egypt).
3) Fecal parameters.
Fecal score was determined using 7 point scale system on fresh faeces weekly. (Bruni et al., 2020; Manchester et al., 2019). Moreover, fecal moisture was determined at the end of the experiment. Fresh faecal samples from all individual dogs in all groups were collected in a plastic bag and stored at 4°C until the transport to the laboratory according to (Bruni et al., 2020).

Statistical analysis:
Data were statistically analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).
All parameters were analyzed via one-way ANOVA using the GLM procedure, except for body condition scores. The overall effect of the treatments was assessed using a model that had diet as the fixed effect and dog pen as the random effect. Body condition score was analyzed using repeated measure procedures, where week was the repeated effect. Significance was set at P ≤ 0.05 and tendency at P ≤ 0.10.

RESULTS

1): Growth performance parameters:
The effect of butyrate and/or postbiotic supplementation on dog growth performance that was expressed by dog body weight, body weight gain, and body condition scoring is shown in (Table 2).

Table (2): Effect of postbiotic and/or sodium butyrate supplementation on dog growth performance and fecal moisture:

<table>
<thead>
<tr>
<th>Item</th>
<th>CON²</th>
<th>BUTR³</th>
<th>POSTB⁴</th>
<th>MIX⁵</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW, Kg</td>
<td>13.00</td>
<td>12.66</td>
<td>13.00</td>
<td>13.33</td>
<td>0.79</td>
<td>0.94</td>
</tr>
<tr>
<td>Final BW, Kg</td>
<td>16.66</td>
<td>18.50</td>
<td>17.93</td>
<td>19.33</td>
<td>0.68</td>
<td>0.10</td>
</tr>
<tr>
<td>ADG, Kg/d⁶</td>
<td>0.08a</td>
<td>0.12ab</td>
<td>0.10ab</td>
<td>0.13a</td>
<td>0.01</td>
<td>0.02*</td>
</tr>
<tr>
<td>BCS</td>
<td>4.80ab</td>
<td>4.71ab</td>
<td>4.67b</td>
<td>4.95a</td>
<td>0.082</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

¹ Values are means.
² Control group fed basal diet
³ Butyrate group fed the basal diet supplemented with coated sodium butyrate (0.5 g/dog/day).
⁴ Post-biotic group fed the basal diet supplemented with postbiotic. (1 capsule/dog/day).
⁵ Mix group fed the basal diet supplemented with mix of postbiotic and coated sodium butyrate. (1 capsule/dog/day and 0.5 g/dog/day respectively).
⁶ Average daily gain.
* Different superscripts within same row are significantly different.

*Different superscripts within same row are significantly different.
The final body weight tended to increase (P=0.10) in the MIX group, followed by the BUTR and POST compared to the control group. On the same pattern, the average daily gain (ADG) increased (P=0.02) in MIX followed by, BUTR, then POST groups compared to the control one. Mix group recorded the best BCS (P = 0.02). Fecal score and fecal moisture composition are shown in (Table 2). The best fecal score was in the MIX group followed by the BUTR one (P = 0.07), While the fecal moisture decreased in all the treatment groups compared to the control one (P = 0.01).

2) Blood indices:
The effect of postbiotic and/or butyrate supplementation on CBC is shown in (Table 3). The CBC was normal in all groups. Some blood metabolites were affected by the postbiotic and butyrate as presented in (Table 4), where the total cholesterol level was the lowest in MIX and BUTR groups (P = 0.01), on the other side, the serum calcium level increased in both MIX and BUTR groups (P = 0.02).

Table (3): Effect of postbiotic and/or sodium butyrate supplementation on fecal moisture and fecal score at the end of the experimental period

<table>
<thead>
<tr>
<th>Item</th>
<th>CON²</th>
<th>BUTR³</th>
<th>POSTB⁴</th>
<th>MIX⁵</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal score</td>
<td>2</td>
<td>2.57</td>
<td>2</td>
<td>2.71</td>
<td>0.23</td>
<td>0.07</td>
</tr>
<tr>
<td>Fecal moisture</td>
<td>60.38a</td>
<td>53.25b</td>
<td>56.60b</td>
<td>54.26b</td>
<td>0.76</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

¹ Values are means.
² Control group fed basal diet
³ Butyrate group was fed on the basal diet supplemented with coated butyrate. (0.5 g/dog/day).
⁴ Post-biotic group fed the basal diet supplemented with postbiotic. (1 capsule /dog/day).
⁵ Mix group fed the basal diet supplemented with mix of post-biotic and coated butyrate. (1 capsule /dog/day and 0.5 g/dog/day respectively).

P-Value for time (week) is 0.01*.
P - Value for interaction (group*week) is 0.48.

Means with different superscripts within row (a,b) are significantly different are P <0.05.

Moreover, the antioxidant activity was assessed in the current study by measuring the level of MDA. The level of MDA tends to decrease (P = 0.09) in MIX and BUTR groups, compared to the other two groups (CON and POSTB) as shown in (Tables 4, 5).
IMPACT OF DIETARY SUPPLEMENTATION OF COATED …………. 

Table (4): Effect of postbiotic and/or sodium butyrate supplementation on dog CBC1 at the end of the experimental period.

<table>
<thead>
<tr>
<th>Item</th>
<th>CON2</th>
<th>BUTR3</th>
<th>POSTB4</th>
<th>MIX5</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC(x×10⁹/L )</td>
<td>18.20</td>
<td>17.73</td>
<td>16.86</td>
<td>17.70</td>
<td>0.96</td>
<td>0.80</td>
</tr>
<tr>
<td>Lymph #/(x×10⁹/L )</td>
<td>4.03</td>
<td>4.33</td>
<td>4.56</td>
<td>3.50</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>Mon#/(x×10⁹/L )</td>
<td>1.03</td>
<td>0.80</td>
<td>1.13</td>
<td>1.00</td>
<td>0.15</td>
<td>0.50</td>
</tr>
<tr>
<td>Gran#/(x×10⁹/L)</td>
<td>14.13</td>
<td>14.33</td>
<td>15.83</td>
<td>15.13</td>
<td>0.70</td>
<td>0.35</td>
</tr>
<tr>
<td>RBC(x×10¹²/L )</td>
<td>7.33</td>
<td>7.29</td>
<td>7.36</td>
<td>7.23</td>
<td>0.22</td>
<td>0.97</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>13.70</td>
<td>13.96</td>
<td>13.53</td>
<td>13.93</td>
<td>0.40</td>
<td>0.85</td>
</tr>
<tr>
<td>HCT %</td>
<td>49.30</td>
<td>50.43</td>
<td>49.30</td>
<td>50.36</td>
<td>1.46</td>
<td>0.90</td>
</tr>
<tr>
<td>MCV fL</td>
<td>67.33</td>
<td>69.26</td>
<td>67.06</td>
<td>69.76</td>
<td>0.99</td>
<td>0.21</td>
</tr>
<tr>
<td>MCH pg</td>
<td>18.66</td>
<td>19.10</td>
<td>18.36</td>
<td>19.23</td>
<td>0.28</td>
<td>0.19</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>27.73</td>
<td>27.63</td>
<td>27.40</td>
<td>27.60</td>
<td>0.16</td>
<td>0.55</td>
</tr>
<tr>
<td>RDW %</td>
<td>15.56</td>
<td>14.93</td>
<td>15.90</td>
<td>15.30</td>
<td>0.27</td>
<td>0.15</td>
</tr>
<tr>
<td>PLT(x×10⁹/L)</td>
<td>400.25</td>
<td>400.66</td>
<td>402.66</td>
<td>406.00</td>
<td>15.98</td>
<td>0.66</td>
</tr>
<tr>
<td>MPV (Fl)</td>
<td>8.86</td>
<td>9.40</td>
<td>9.43</td>
<td>9.23</td>
<td>0.35</td>
<td>0.67</td>
</tr>
</tbody>
</table>

1 Values are means, CBC= complete blood count.  
2 Control group fed basal diet.  
3 Butyrate groupfed the basal diet supplemented with coated butyrate. (0.5 g/dog/day).  
4 Post-biotic group fed the basal diet supplemented with postbiotic. (1 capsule /dog/day).  
5 Mix group fed the basal diet supplemented with mix of postbiotic and coated butyrate (1 capsule /dog/day and 0.5 g/dog/day respectively).

Table (5): Effect of post-biotic and/or Butyrate supplementation on dog selected serum parameters1 at the end of the experimental period.

<table>
<thead>
<tr>
<th>Item</th>
<th>CON2</th>
<th>BUTR3</th>
<th>POSTB4</th>
<th>MIX5</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>233.28</td>
<td>171.53</td>
<td>211.07</td>
<td>186.01</td>
<td>9.86</td>
<td>0.01*</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>12.80</td>
<td>15.13</td>
<td>12.34</td>
<td>15.34</td>
<td>0.64</td>
<td>0.02*</td>
</tr>
<tr>
<td>MDA6 (nmol/ml)</td>
<td>188.84</td>
<td>165.07</td>
<td>183.38</td>
<td>160.79</td>
<td>7.78</td>
<td>0.09</td>
</tr>
</tbody>
</table>

1 Values are means.  
2 Control group fed on basal diet.  
3 Butyrate groupfed the basal diet supplemented with coated butyrate. (0.5 g/dog/day).  
4 Post-biotic group fed the basal diet supplemented with postbiotic. (1 capsule /dog/day).  
5 Mix group fed the basal diet supplemented with mix of post-biotic and coated butyrate (1 capsule /dog/day and 0.5 g/dog/day respectively).  
6 Malondialdehyde.  
*Different superscripts within same row are significantly different (P ≤ 0.05).
DISCUSSION

Butyrate is a short-chain fatty acid that is getting importance and popularity due to its remarkable action in enhancing general health and growth indices in several animal species (Badejogbin et al., 2019; Bolívar Ramírez et al., 2017; Donohoe et al., 2011; Gao et al., 2009; Izuddin et al., 2019; Lan et al., 2020; Liu et al., 2021; Scarpellini et al., 2007; Zhang et al., 2011; Zou et al., 2019). However, there is limited data concerning the effects of sodium butyrate and or post-biotic on the growth performance, antioxidant activity, cholesterol level and calcium absorption in dogs.

In this study, the results showed that there is improvement in final body weight, average daily gain and BCS noticed in dogs receiving butyrate or butyrate combined with post-biotic compared to the control group. The results in this study indicated that dietary sodium butyrate supplementation especially when combined with postbiotic could improve the growth performance of dogs. The following are some proposed mechanisms for improving growth performance: Sodium butyrate has a special role in activating pepsin and other digestive enzymes, promoting the proliferation of intestinal villus, and regulating the structures of gut bacterial community, resulting in the improvement of ADG, final body weight and BCS. (Guilloteau et al., 2010); sodium butyrate, which is an important source of energy for intestinal epithelial cells, decreases the ratio of amino acids and glucose consumed by intestinal epithelial cells, consequently more nutrients could be used for animal growth (Sun et al., 2020; van der Meulen et al., 1997).

In addition, a fecal score of 3 or less is considered to be normal (Fenimore et al., 2017; Niina et al., 2019; Tupler et al., 2012). In the present study, butyrate and postbiotic combination also enhanced fecal score and decreased fecal moisture percent to an optimum level compared with the other groups in the experiment indicating better nutrient absorption from the gut (Weber et al., 2002). However, a complete digestibility trial was not performed in this investigation. There were also some reports that dietary sodium butyrate supplementation have no effects on faecal characteristics of dogs (Hesta et al., 2008). The causes for these inconsistent results may be related to the added dose of sodium butyrate and the short experimental period serum calcium level increased in dogs that received butyrate alone or in combination with post-biotic. This could be postulated by the effect of butyrate on calcium absorption and transport in the intestine, where sodium butyrate regulates the expression of genes involved in active intestinal calcium absorption (Gommers et al., 2022).
The beneficial decrease in total cholesterol level of dogs received butyrate and butyrate-postbiotic mix in the current study is similar to (Yu et al., 2017). This decrease was formerly reported due to the global effect of butyrate to downregulate the expression of key genes involved in intestinal cholesterol biosynthesis, potentially inhibiting this pathway. (Alvaro et al., 2008; Canani et al., 2011).

The study of the effect of butyric acid or its sodium salt on antioxidant capacity is limited, especially in dogs. The results of the present study declared that dietary sodium butyrate supplementation could improve serum antioxidant biomarker especially when combined with post-biotic, as the level of MDA tends to decrease in mix and butyrate group. These results agree with study of (Zhang et al., 2011) who clarified that dietary sodium butyrate addition declined the level of MDA. Butyrate enhances antioxidant properties and retards damage of the mucosa by scavenging free radicals, where decreased MD Aconcentrations (Wu et al., 2018). The antioxidant property of butyrate remains unknown, therefore, it needs more interest and further studies.

CONCLUSION

From the present study it can be concluded that butyrate could be used alone or in combination with postbiotics to enhance growth performance, nutrient absorption, antioxidant status and the general health of dogs. Moreover, butyrate and/or postbiotics had an impact on dog metabolism that resulted in inhibition of cholesterol biosynthesis, which reflected positively on dog health. Further studies should be focused on the mechanism by which butyrate and/or postbiotics affect positively health and performance of dogs.

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Conflict of interest:
The authors declare that they have no conflicts of interest to disclose.

REFERENCES


