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Biochemical and Fecal Evaluation of Digestive and Absorptive Function in Dogs Fed on Different Feed Manner

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Abstract

In the present study were used Total 40 adult male aged around 3-4 years and weight range 28 -35 kg which belong to three categories, the first category are canine dogs that use for security duties K9 (N=5). The second category local dogs (morgel dogs) obtained from Karmat ali area (Basrah) were used as before and after adapted (N=5). The third category is represent dogs breed in homes, and selected through screening of 30 dogs in veterinary clinic of Basrah province to choose only five dogs (N=5) which suffered from chronic diarrhea, weight loss or vomiting. Both 1 and 3 groups were given the same food administrated dry food (Diamond -HI-ENERGY); while, the 2 and 4 groups were given different food administrated. The physiological parameters were measured at end of an experiment, and the blood serum samples were collect in addition to anthropometric examination. The physiological parameters included an evaluation ALT, AST, ALP and TSB, urea, creatinine and digestive enzymes concentration (amylase, lipase and tyrosine) in addition to fecal testing (fat, starch, pH and occult blood) were done. The result of biochemical analysis was showed a significant increase in ALT, AST, ALP and TSB activity in dogs of group 4 which suffering from diarrhea and / or weight loss when compared with other study groups. The renal function parameters included urea and creatinine was increased significantly ($p<0.05$) in morgel dogs when compared to other study groups. Whereas, creatinine was decreased significantly ($p<0.05$) in group 3 of morgel dogs after adaptation on standard feed compared to all study groups. However, biochemical analysis was revealed a significant increase ($p<0.05$) in concentration of digestive enzymes (amylase, lipase and tyrosine). In conclusion, there was many cases of dogs that received in clinical veterinary medicine suffered from diarrhea, vomiting or weight loss belong to exocrine pancreatic insufficiency due to decreasing the values of measured amylase, lipase and tyrosine. Also, the fecal examination referred to exocrine pancreatic insufficiency due to fecal starch and fecal fat showed in the stool examination of dogs received in clinical veterinary medicine suffered from diarrhea, vomiting or weight loss as compared with other study groups.

Keywords: Pancreas, Creatinine, Amylase, Lipase, Tyrosine

Introduction

Dogs have various medical conditions that come to veterinary clinics, mostly with symptoms such as diarrhea, vomiting, and weight loss. Breeders complain that these dogs although they

eat food periodically and of good quality but have not shown improvement and suitable growth. The importance of these animals at the present time and their use in different purpose including their use in security companies, the detection of narcotic substances in the police dog unit, as well as the breeding of decorative dogs in homes, especially in Iraq, where interest in these animals has increased recently. The digestive system has very important main functions; Digestion and absorption are both activities of the digestive system. Food is broken down into individual particles small enough to be absorbed through the intestinal wall during digestion (Guo et al 2017). Absorption is defined as the process of transferring nutrients from the digestive system (GI) into bloodstream (Kiela and Ghishan 2016). The process of digestion and absorption are linked with each other, as food is not used if these two processes do not occur, and one of the most important diseases that cause maldigestion and malabsorption is exocrine pancreatic insufficiency (Bruno 2001). Some studies have been conducted regarding the inefficiency of the external pancreas are caused by maldigestion and malabsorption that cause many symptoms, including diarrhea, vomiting and weight loss and it is known that the digestion or metabolism process will not be completed unless the two processes of digestion and absorption done in the same manner (Krishnamurty et al 2009, Wiberg 2012, Maddison et al 2015).

As well as, Singh et al (2018) found that the cases of digestive symptoms indicated that the main reason behind this condition is exocrine pancreatic insufficiency or pancreatic insufficiency. Where, the pancreas is one of the organs of the body that has both secretory and endocrine functions and exocrine pancreatic insufficiency secrete an insufficient amount of essential digestive enzymes namely amylase, lipase and serum tyrosine like immune reactivity which lead to malabsorption and maldigestion that clinically manifested in loss of appetite, digestive disorders, weight loss, and loss of general body condition (Barrouin-Melo et al 2018). The clinical signs caused by small intestinal maldigestion and malabsorption include polyphagia, coprophagia, weight loss, chronic diarrhea with frequency 3-8 per day. Therefore, this information tends an attention to study digestive system functions in dogs due to the lack of accurate data, so this study was conducted.

Material And Methods

Animal study

Total 40 adult male dogs were use in the present study aged around 3-4 years and weight range 28 -35kg which belong to three categories (each of five) dogs categories; K9, mongrel dog which used as before and after adapted and dogs brought up at home screening of 30 dogs in veterinary clinic of Basrah province to choose only five dogs (N=5) that suffered from chronic diarrhea or weight loss or vomiting, maldigestion and malabsorption. Male aged between 3-4 years and weighted between 28 -35 kg were used. Then the mongrel dog animals were adapted for a period of three weeks under the same environmental conditions as the police dog unit in Basra Police Directorate and after adaptation period, this group was given the same food administrated dry food (Diamond -HI-ENERGY), (Table 1).

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Table 1. Composition of Diamond-HI-ENERGY dry-food

Composition	**%**
Crude protein	26.0 % minimum
Crude fat	18.0 % minimum
Crude fiber	3.0 % minimum
Moisture	10.0 % minimum
DHA (Docosahexaenoic acid)	0.05 % minimum
Calcium	1.2 % minimum
Phosphorus	1.0% minimum
Zinc	150 mg/kg minimum
Selenium	0.35 mg/kg minimum
Vitamin E	150 IU/kg minimum
Vitamin A	15,000 IU/kg minimum
Omega-6-fatty acids	3.0 % minimum
Omega-3-fatty acids	0.5 % minimum
Total Microorganisms	Not less than 80,000,000 CFU/IB

Blood collection

Ten ml of venous blood were drained from the cephalic of all dogs using disposable syringe with a 20 gauge needle. Then blood divided equally into a gel and clot activator tubes, and kept at room temperature to analysis of ALT, AST, ALP, TSB, urea, creatinine, tyrosine-like immune-reactivity, lipase and amylase.

Feces sample

The samples were collected from dogs carefully to prevent contamination of sample using sterile swab stick sterilized by Ethylene and feces, and from rectum by using class rod (Uchoa et al 2017).

Statistical analysis

It conducted in SPSS (*version* 13) Software by ANOVA. The detection limit for each FOB test was set depending on the blood levels measured. Differences were considered significant at $P<0.05$ (Al-Gharban 2017, Gharban et al 2022).

Results

Biochemical parameters in adult dogs of different food consumption

The biochemical analysis for the studied groups of adult dog sera that fed on different food pattern and habits in Basrah province (Table 2).

Table 2. Biochemical parameters of liver enzymes activities and renal function parameters in dogs of different food consumption

Group	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**	**TSB (mg/d)**	**Urea (mg/dl)**	**Creatinine (mg/dl)**
G: 1	42.02 ± 2.03 B	58.72 ± 8.85 B	38.64 ± 7.00 D	0.48 ±0.0B	44.4± 5.5 BC	1.22 ± 0.15 B
G: 2	47.76 ±	58.86 ±	51.86 ±	0.5 ±	62.30± 3.76	1.39 ± 0.10

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	8.44 B	8.85 B	2.95 B	0.03B	A	A
G: 3	46.08 ± 3.13 B	61.02 ± 5.09 B	43.62 ± 3.26 C	0.4 ± 0.06B	37.6 ± 4.16C	1.03 ± 0.16 C
G: 4	69.92 ± 7.06 A	70.68 ± 7.70 A	74.40 ± 4.84 A	0.68 ±0.1A	47 ± 5.78 B	1.32± 0.1 AB

Variation in large letters refer to significant difference (P<0.05)

The measurement of liver enzymes activities and renal function parameters are vital indicators for the health of animal. The study results revealed a significant increase (p<0.05) in ALT activity in dogs of group 4 that suffering from diarrhea and/or weight loss when compared with other studied groups. In contrast, AST activity also showed significant increase (p<0.05) in dogs suffering from diarrhea and/or weight loss (group 4) when compared with other studied dogs groups that fed on different food pattern (groups 1-3). The ALP activity enzyme value showed significant elevation (p<0.05) in dogs suffered from diarrhea and/or weight loss or vomiting (group 4) compared to all other studied groups. While, dogs that breed in the street (group 2) showed significant increase values for ALP activity than values of local dogs after adaptation and dogs of police station which appeared less significant values for ALP activity when compared with other studied groups. Also, TSB that revealed significantly (p<0.05) increased in group 4 of dogs when compared to other study groups. The renal function parameters urea and creatinine revealed significantly (p<0.05) increase in morgel dogs of dogs when compared with other studied groups. Whereas, creatinine showed significantly (p<0.05) decreased in group 3 for morgel dogs after adaptation on standard feed compared to all studied groups.

Digestive enzymes concentration in dogs of different food consumption

The digestive roles of (amylase, lipase, and tyrosine) in various dog feed ways were reported. When compared to the other dog groups investigated, dogs in group 4 showing evidence of digestion and absorption had a significant decline (p<0.05) in amylase concentration. When compared to other study dogs while lipase concentration appeared significant decrease when compared to other groups. Also, lipase concentrations of local dogs of group 2 were higher (p<0.05) than other studied groups. The results showed significant increase (p<0.05) in the concentration of tyrosine enzyme in local dogs group before adaptation when compared to other studied groups that feed in different methods (Table 3).

Table 3. Biochemical parameters enzymes activities Amylase, Lipase and Tyrosine in dogs of different food consumption

Group (G)	Amylase U/L	Lipase U/L	Tyrosine mg/dl
G: 1	75.40±13.02B	38.20± 9.97 B	5.23 ± 1.56 B
G: 2	90.54 ± 16.43 A	93.28 ± 13.4A	14.44 ± 1.43 A
G: 3	74.86 ± 14.08 B	43.24 ± 7.08B	8.05 ± 3.19 B
G: 4	53.40 ± 9.50 C	25.64 ± 6.13C	6.05 ± 1.42 B

Variation in large letters refer to significant difference (P<0.05)

Fecal occult blood and pH in dogs of different food consumption

The examination of feces for PH and occult blood are an important tool to diagnosis the

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disorder of GI function .Therefore, shifting of acidity in PH stool of dogs for all studied groups. Despite of acidity of feces in all dogs, there was increase in PH acidity in group 4 of dogs that suffered from diarrhea and weight loss compared to other dogs in studied groups. The fecal occult blood test showed negative marks for the group of K9 dogs and in morgel dogs after adaptation on standard feed in their stool test. Whereas, the most of morgel dogs before adaptation and dogs that suffered from GI disorder showed positive marks in their stool examination (Table 4).

Table 4. Fecal PH and fecal occult blood test in dogs of different food consumption

Groups(G)	pH(mean)	FBO
G: 1 N=5	6.6	5 dogs= Negative
G: 2 N=5	6.2	4 dogs= Positive 1 dog= Negative
G:3 N=5	6.4	5 dogs = Negative
G:4 N=5	4.8	5 dogs= Positive

Fecal starch and fecal fat examination

The findings of these parameters were detailed (Figures 1-8).

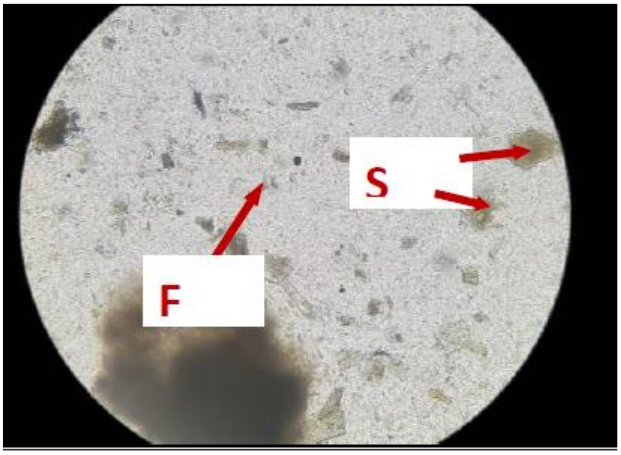

Fig. 1. Fecal picture of K9 group represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

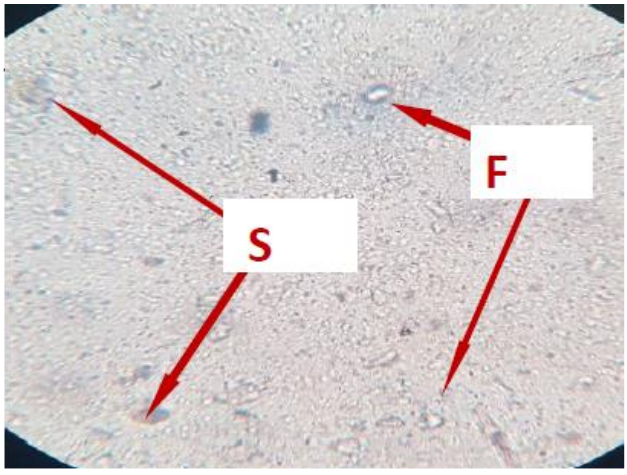

Fig. 2. Fecal picture of K9 group represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

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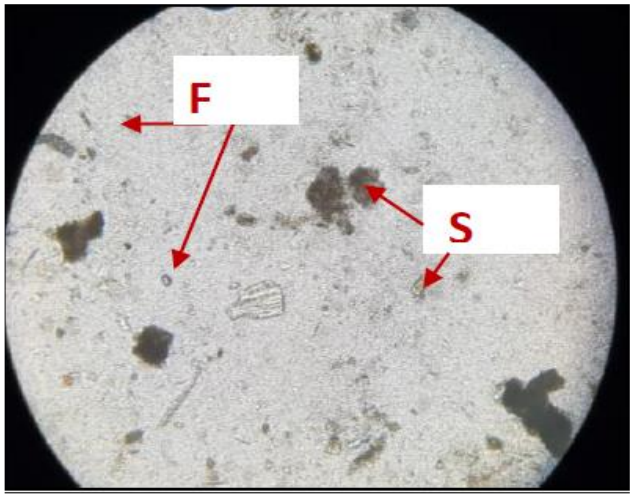

Fig. 3. Fecal picture of morgel dog group before adaptation represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

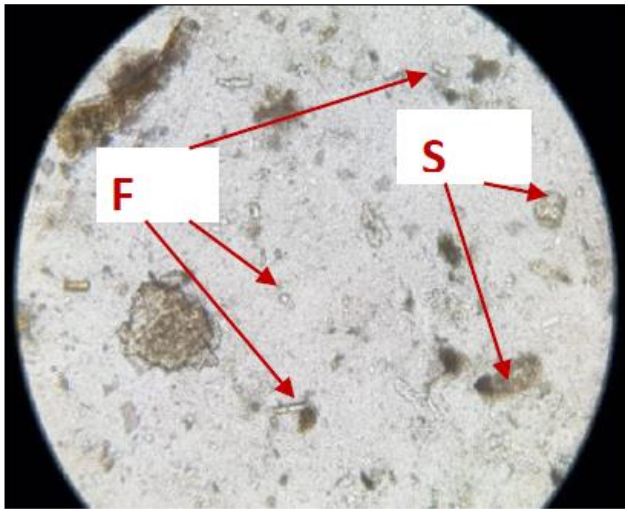

Fig. 4. Fecal picture morgel dog group before adaptation represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

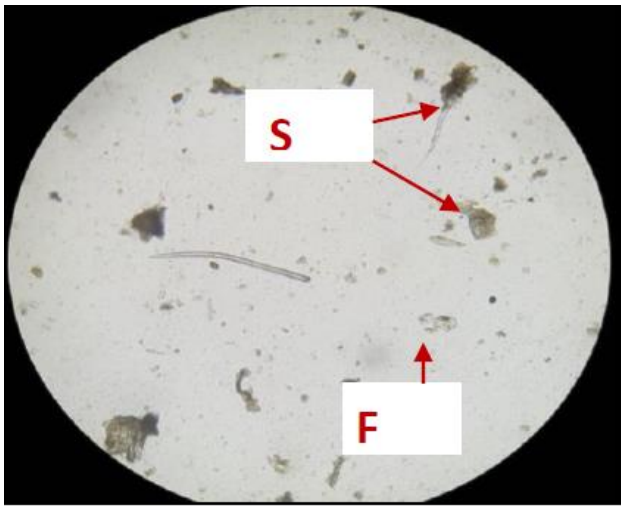

Fig. 5. Fecal picture morgel dog group after adaptation represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

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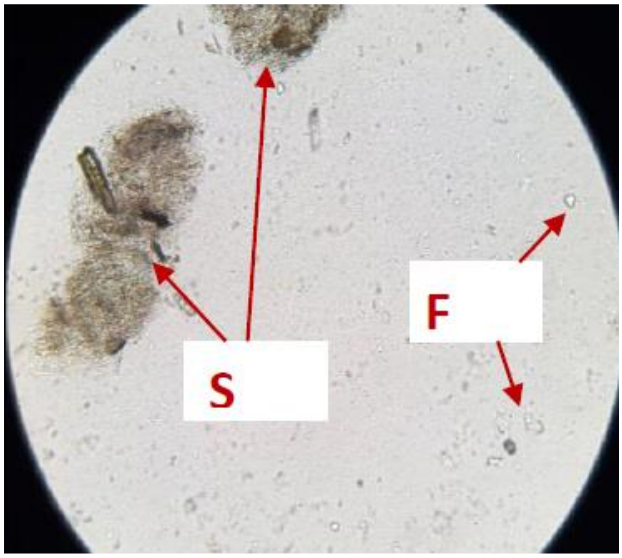

Fig. 6. Fecal picture morgel dog group after adaptation represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

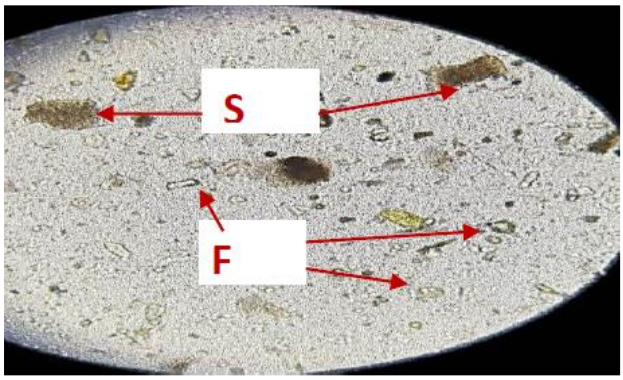

Fig. 7. Fecal picture home dog represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

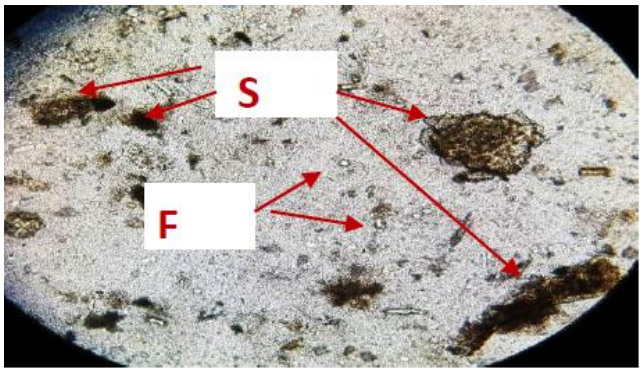

Fig. 8. Fecal picture home dog group represent fecal fat (F) and fecal starch (S) X400, Hematoxiline and eosin stain

Discussion

Biochemical analysis is a very helpful tool to evaluate the health status of animals. In this study, the levels of liver function enzymes measured to evaluate the effect of different food consumption in study animals as well as serum concentrations of compounds that are

normally removed from the circulation by liver and then processed or expelled via the biliary system (e.g., bilirubin, bile acids, ammonia, cholesterol) and chemicals that are normally generated by the liver are measured in liver function tests (e.g., albumin, globulins, urea, cholesterol, coagulation factors). Although abnormal blood concentrations of these substances can result from nonhepatic factors, the detection of abnormal concentrations in addition to evidence of liver injury as detected by changes in leakage or induced enzyme activities can supply further evidence of significant liver disease or liver failure. Often, however, liver biopsy is required for a definitive diagnosis (Thrall et al 2012). Liver function tests include: alanine transaminase (ALT) is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into blood stream and levels increase, previously referred to as serum glutamic pyruvic transaminase (SGPT) is a leakage enzyme that is free in the cytoplasm. In dogs, the highest concentrations of ALT occur in hepatocytes (especially those in periportal regions), and the ALT assay is commonly included in the serum biochemical profiles of these species (Boyd 1983). In other cases, ALT activity is the only test available used to detect hepatocyte injury in dogs because ALT is much more liver specific than AST. However, ALT is not totally liver specific; severe muscle damage or disease can cause increases in serum ALT activity (Swenson and Graves 1997). ALT activity of muscle is less than that of the liver (activities in skeletal and cardiac muscle are approximately 5% and 25% that of liver activity, respectively. Increased serum ALT activity in dogs usually indicates hepatocyte death or sublethal hepatocyte injury; however, necrosis or sublethal muscle cell damage must also be addressed (Boyd 1983).

While, aspartate aminotransferase (AST), previously termed serum glutamic oxaloacetic transaminase (SGOT), hepatocytes and muscle cells (both skeletal and cardiac) of all animals have the largest amounts (Boyd 1983). AST is an enzyme that aids in the metabolism of amino acids, which are generally present in small amounts in the blood. An increase in AST levels could be a sign of liver disease, illness, or muscle damage. As a result, AST isn't just a liver enzyme. AST is a leaking enzyme that is primarily found in the cytoplasm, as approximately 20% of the cells are found within mitochondria (Keller 1981). Hepatocyte AST in contrast to ALT is found in the highest concentration in cells of the periacinar area, which surrounds central veins (Center 2007). Increased serum AST activity can be caused by hepatocyte or muscle cell injury that can be potentially lethal or sublethal. In dogs serum AST activity will increase as a result of the same liver diseases previously listed for ALT and generally parallels ALT activity, but the magnitude of the increase may be less than that of ALT (Center 2007). Following acute liver injury in some animals, serum AST activity may recover to baseline quicker than ALT, making repeated assessments valuable for disease resolution monitoring (Center 2007). Despite the fact that AST is less liver specific than ALT, it may be more sensitive in diagnosing some liver illnesses in dogs. Similar to ALT, mild increases in AST activity may be seen in dogs as a result of enzyme induction due to corticosteroids and possibly phenobarbital, although there is some controversy because muscle is a possible source of serum AST activity, measurement of an enzyme specific for muscle injury is useful to determine if the increase in AST activity is due to muscle injury (Muller et al 2000, Krause et al 2003, Webster 2005). As well as, Many tissues, including the liver, bone, kidney, gut, pancreas, and placenta, produce alkaline phosphatase (ALP), an induced enzyme linked to cell membranes (Fernandez and Kidney 2007). ALP is an enzyme

that breaks down proteins and is found in the liver and bones. ALP levels that are higher than usual may suggest liver damage or disease, such as a clogged bile duct, or certain bone diseases. The intestinal isoenzyme can also undergo ALP in the liver is associated with biliary epithelial cells and canalicular membranes of hepatocytes (Center 2007). Increased blood ALP activity can cause increasing enzyme synthesis, bile salt solubilization of membranes, and release of membrane blebs after cell injury in a number of hepatobiliary disorders (Fernandez and Kidney 2007). In dogs, cholestatic disorders may elevate significantly serum ALP activity (Thrall et al 2012). The current biochemical results showed a highly significant values increase in the serum activity of (AST, ALT and ALP) for dogs brought up at home (G: 4) that collecting samples from the veterinary clinic suffering diarrhea or weight loss comparative to other groups.

Also, TSB that revealed significantly ($p<0.05$) increase in group 4 of dogs when compared with other studied groups. These results in agreement with Adamama-Moraitou (2004) revealed a significant increase in ALT, AST and ALP enzymes activities in dogs that suffered from exocrine pancreatic insufficiency, and he mentioned that the consequences of canine EPI on liver structure and function are not well established. It has been reported that the serum activity (ALT and ALP) were consistently elevated in dogs with acute liver disease and develop with hepatocellular necrosis and inflammation. The incidence of abnormally elevated liver enzyme activity is far higher than frequency of liver disease. This has to do with the impact of systemic diseases on the liver. The liver, as a sentinel organ between the alimentary canal and the systemic circulatory system, is exposed to wide range of toxins, drug metabolites, endotoxins, and infectious agents. Consequently, a wide spectrum of nonhepatic disorders may influence liver enzyme activity (Center 2007). The current biochemical parameters of liver enzymes activities and renal function parameters in dogs of different food consumption results of significant increases of liver function enzymes in dogs of different food consumption were in agreement with our hematological investigations that showed significant increase in lymphocyte count as indication to inflammation in the body. The spectrophotometric assays for urea and creatinine measurements in serum of dogs showed the urea value revealed significantly increase in mongrel dogs (G: 2) when compared with other studied groups. Also, creatinine that revealed significantly increase in G: 4 for the dogs that collected samples from the veterinary clinic to appear significantly increase in creatinine when compared to other studied groups. These results may agree with Ramazan et al (2020) as mongrel dogs aged 1 to 7 years had higher serum urea and creatinine levels, according to the authors. They were tested for kidney disease and other abnormalities using clinical and biochemical methods.

The digestive roles of amylase, lipase and tyrosine in different feed methods in dogs recorded a significant decrease in amylase, lipase and tyrosine for the dogs brought up at home (group 4) collecting samples from the veterinary clinic for animals suffering from diarrhea or weight loss when compared to other study groups. Therefore this results were in agreement with Watson (2003) who found a low levels of amylase, lipase and tyrosine in serum of dogs (case 2) was a seven-year-old, neutered female Gordon setter who had been diagnosed with diabetes one year prior to presentation and had been initially stable on isophane insulin once daily and high-fiber diet (Hill's Pet Nutrition's Prescription Diet Canine w/d). EPI indicates permanent damage to pancreatic cells that reduce lipase, tyrosine and amylase-producing

(Garg and Singh 2019).

Also, Steine et al (2006) study divided dogs into two groups; first group comprised healthy dogs obtained from different sources that previously used to establish a reference range for serum lipase concentration. While, second group included dogs suffered from exocrine pancreatic insufficiency that had been enrolled in an unrelated study. Weight loss, diarrhea, and a serum lipase activity assay were used to confirm the diagnosis of exocrine pancreatic insufficiency in those dogs. The results showed that all dogs with exocrine pancreatic insufficiency had serum lipase values less than the lower limit of the reference range. This is consistent with our findings, in which lipase concentration was shown to be significantly lower ($p<0.05$) in group 4 when compared to the other dog groups studied. Local dogs (group 2), on the other hand, showed a substantial rise ($p<0.05$) in yrosine and lipase enzyme concentrations before adaption. When compared to other studied groups that feed in different methods. Enzymatic assays that measure serum lipase activity detect lipase from pancreas as well as other tissues. Thus, increases in serum lipase activity are not specific for pancreatic injury (Hulsebosch et al 2016). In these species, although there are rare reports of increased serum lipase activity is associated with acute pancreatitis. The activity of serum lipase in dogs can be used as a screening test for pancreatitis and frequently included on standard biochemical profiles. Other than pancreatitis, elevated serum lipase activity can be because of decreased renal excretion and/or lipase inactivation, dogs with prerenal, renal, or postrenal azotemia may have higher serum lipase activity (Thrall et al 2012).

Gilson et al (1990) described the test of occult blood as a simple and available test for in-practice use, which detects the pseudoperoxidase activity of fecal hemoglobin and pickup minute amounts of fecal blood at concentrations as low as 20× to 50× times less than those where blood is visible grossly. A loss of 30–50% of blood volume into GI tract can occur without gross blood being visible in feces. The test procedure involves application of feces to test paper when blood is present, and peroxidase activity results in formation of blue color. Blood from upper GI tract is usually digested but not always grossly visible in feces, but blood from the lower GI tract is undigested, and is normally evident grossly. Loss of large amounts of blood in the upper GI tract can cause rapid transit times, and occasionally, results in grossly visible blood in the feces. Rice and Ihle (1994) studied effects of diet on fecal occult blood testing in healthy dogs. As well, the results showed positive FOB among the dogs that suffered from diarrhea, weight loss or vomiting (4) and also in group 2 of morgel dogs (Table 5). A diagnostic fecal occult blood test is described in several veterinary publications as being used in the search for a diagnostic test for intestinal bleeding.

These results were substantially identical to the ones previously described (Pierini et al 2020). The low rate of positive results may be related to the difference in test sensitivities between the Hem-occult test and other FOBts, according to those who mention it, rather than the different diets used together with the materials and methods also effect on FOB results. Also, the result found that pH of canine excrement is also varying, although frequently alkaline, according to the findings. The pH of a healthy group is 6.6 on average. This finding was consistent with Rentas et al (2020) who found that the prebiotics evaluated at their respective concentrations had no effect on fecal pH. A stool pH level of 4.8 was found in group 4 with diarrhea and weight loss. Where, An acidic stool can indicate a digestive problem such as lactose intolerance, an infection such as E. coli or Rotavirus, or an overgrowth of acid-

producing bacteria such as lactic acid bacteria (Beasley 2004). The presence of reducing substances indicates carbohydrate intolerance, which is usually secondary to viral illness and transient in nature. Leukocytes, primarily neutrophils are shed into stool during Enteroinvasive infections of the large bowel. These results were nearly similar to those mentioned Algya et al (2018) who mentioned that dogs fed RAW had the lowest fecal pH.

The present study appeared there are inordinate amount of fat in the feces. It could indicate a problem with absorption. This indicates that the body isn't absorbing nutrients properly or isn't producing the enzymes or bile required for proper digestion. However, Steatorrhea may be caused by bacterial or parasitic infections in gastrointestinal tract. These results were nearly similar to those mentioned (Isaiah et al 2017). The fecal microbiome of dogs with exocrine pancreatic insufficiency; fat malabsorption and consequently they are excreted in the feces as fat droplets which explain. Also, Kim et al (2019), who mention that fecal fat excretion is influenced by body weight. However, evaluation of microscopically changes of fecal starch and fecal fat in dry-extruded canine diets. According to (Sandri et al 2020, Algya et al 2018, Beloshapka et al 2011, Bermingham et al 2017, Kim et al 2017, Sandri et al 2016). In feces of dogs, the starch-to-lipid ratio in the diet caused a shift in microbial communities. Several other factors, such as the extent of food thermal treatments and the administration of raw meat, prebiotics, and protein source, all have an impact on gut microbiome, according to recent researchers (Sandri et al 2019). The digestibility of starch in the ileum varies depending on the starch source, amount, and processing parameters. By stimulating colonic bacterial fermentation, starch that escapes duodena-ileal digestion can affect fecal quality. The goal of this study was to see how different resistant starch (RS) sources and levels affected the fecal score of dogs of various breeds and sizes.

Conclusion

According to the study design and results, the study can conclude there was many cases of dogs that received in clinical veterinary medicine suffered from diarrhea or vomiting or weight loss belong to exocrine pancreatic insufficiency due to lowered values of measured amylase, lipase and tyrosine. Also, the fecal examination referred to exocrine pancreatic insufficiency due to fecal starch and fecal fat that showed in the stool examination of dogs received in clinical veterinary medicine suffered from diarrhea or vomiting or weight loss and compared with other studied groups.

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