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TOPICAL REVIEW

The importance of methane breath testing: a review

B P J de Lacy Costello¹, M Ledochowski² and N M Ratcliffe¹

¹ University of the West of England, Institute of Biosensor Technology, Coldharbour lane, Frenchay, Bristol BS16 1QY, UK
² Academy of Clinical Nutrition Anichstrasse, 17, A-6020 Innsbruck, Austria

E-mail: Norman.Ratcliffe@uwe.ac.uk

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Abstract
Sugar malabsorption in the bowel can lead to bloating, cramps, diarrhea and other symptoms of irritable bowel syndrome as well as affecting absorption of other nutrients. The hydrogen breath test is now a well established noninvasive test for assessing malabsorption of sugars in the small intestine. However, there are patients who can suffer from the same spectrum of malabsorption issues but who produce little or no hydrogen, instead producing relatively large amounts of methane. These patients will avoid detection with the traditional breath test for malabsorption based on hydrogen detection. Likewise the hydrogen breath test is an established method for small intestinal bacterial overgrowth (SIBO) diagnoses. Therefore, a number of false negatives would be expected for patients who solely produce methane. Usually patients produce either hydrogen or methane, and only rarely there are significant co-producers, as typically the methane is produced at the expense of hydrogen by microbial conversion of carbon dioxide. Various studies show that methanogens occur in about a third of all adult humans; therefore, there is significant potential for malabsorbers to remain undiagnosed if a simple hydrogen breath test is used. As an example, the hydrogen-based lactose malabsorption test is considered to result in about 5–15% false negatives mainly due to methane production. Until recently methane measurements were more in the domain of research laboratories, unlike hydrogen analyses which can now be undertaken at a relatively low cost mainly due to the invention of reliable electrochemical hydrogen sensors. More recently, simpler lower cost instrumentation has become commercially available which can directly measure both hydrogen and methane simultaneously on human breath. This makes more widespread clinical testing a realistic possibility. The production of small amounts of hydrogen and/or methane does not normally produce symptoms, whereas the production of higher levels can lead to a wide range of symptoms ranging from functional disorders of the bowel to low level depression. It is possible that excess methane levels may have more health consequences than excess hydrogen levels. This review describes the health consequences of methane production in humans and animals including a summary of the state of the art in detection methods. In conclusion, the combined measurement of hydrogen and methane should offer considerable improvement in the diagnosis of malabsorption syndromes and SIBO when compared with a single hydrogen breath test.
1. Introduction

Malabsorption of sugars can lead to symptoms which reduce the quality of life of sufferers. For instance more than 50 million Americans cannot adequately hydrolyze lactose. This can lead to symptoms of non-ulcerative dyspepsia and irritable bowel syndrome, such as bloating, diarrhea, flatulence, abdominal cramps and severe discomfort [1].

The malabsorption results in hydrogen and methane being produced in the digestive system mainly by the bacterial fermentation of carbohydrates (sugars, starches and vegetable fibers). The generation of these gases in the gut results in some gas transfer through the intestinal wall into the blood stream and then to the lungs, from where they can be quantitatively measured. However, the gases mostly remain in the intestine where accumulation can occur giving rise to abdominal bloating or distension. Distension can cause abdominal pain. Some of the increased amounts of gas are passed as flatus.

Malabsorption of sugars in the small intestine (where there are normally few bacteria) results in their passage to the large intestine (where there are very high concentrations of bacteria). This results in increased bacterial numbers and gas production which can push bacteria back into the small intestine as the ileocecal valve becomes insufficient to cope with the increasing intraluminal pressure. Bacteria in the small intestine, when present in large numbers, can compete with the human host for the food that is eaten. This can lead to vitamin and mineral deficiencies. In advanced cases of small intestinal bacterial overgrowth (SIBO), the bacteria use up enough food that there are insufficient calories for the host, thereby leading to malnutrition. The symptoms of fructose malabsorption (which may affect approx. 30% of the European population) for instance are characterized by the inability to absorb fructose in the small intestine leading to bloating, cramps, osmotic diarrhea and other symptoms of irritable bowel syndrome which can be seen in about 50% of fructose malabsorbers [2]. Low serum tryptophan and signs of folic acid deficiency can also be linked with the inability to absorb fructose efficiently [3].

SIBO has been causally linked to a number of health issues [4] including depression [5] and has been associated with an increased immune activation [3]. It should be noted that there are other reasons for SIBO apart from sugar malabsorption. The excess bacteria also convert food including sugar and carbohydrate into substances that can be irritating or toxic to the cells of the inner lining of the small intestine and colon. These irritating substances e.g. excess short chain fatty acids produce diarrhea (by causing secretion of water into the intestine). There is also some evidence that the production of methane causes constipation by reducing peristalsis [6]. However the fact that microorganisms can produce gas (particularly hydrogen and methane) means that this can be used to aid in the diagnosis of SIBO.

Malabsorption is typically determined by ingesting the sugar of interest e.g. lactose and determining breath hydrogen over time. The level of hydrogen in alveolar air will rise significantly within 1–2 h (depending on the intestinal transit time) only if the sugar is not digested and therefore reaches the colon. False-negative results are reported to be from 2.5% to 15% of all lactose malabsorbers due to a variety of causes [7] and work by Lee stated that 8% to 12% of all patients tested for lactose intolerance will be false negative if only hydrogen is measured [8]. Many of the false-negative reports can be avoided by measuring methane in addition to hydrogen as methane is produced at the expense of hydrogen because of methanogenic flora converting colonic hydrogen into methane [9].

SIBO is diagnosed differently. The patient takes a dose of carbohydrate such as lactulose (typically 10 g) or glucose (typically 50 g) and samples of breath are analyzed for hydrogen, typically every 15–20 min for up to 3 h. Where the patient is administered glucose a rise in hydrogen concentration, typically > 10 ppm above the baseline level is indicative of a positive test [10]. Lactulose is a sugar that is digested by colonic bacteria and not by the human host. The ingested lactulose should pass through the small intestine undigested and reach the colon where the bacteria produce gas. In the normal individual, there is a single peak of gas in the breath following the ingestion of lactulose when the lactulose enters the colon. Individuals with SIBO may produce two significant peaks of gas in the breath. The first abnormal peak occurs as the lactulose passes the gas-producing bacteria in the small intestine, and the second normal peak occurs as the lactulose enters the colon. If the baseline levels of hydrogen rise by >20 ppm after ingestion of lactulose, this can also indicate a positive test [10]. Recently, a number of studies [11–13] have demonstrated the limitations of the use of lactulose in diagnosing SIBO, mainly because of the high rate of false positives. Hydrogen breath testing may be able to diagnose only 60% of patients with SIBO. A major problem is that there is no ‘gold standard’ for the diagnosis of SIBO since culture of the bacteria has its own limitations. There has been much less work undertaken on combined methane/hydrogen detection for improving SIBO diagnoses. This is most likely in part because until recently, the only methane analysis equipment was expensive and needed skilled operatives.

This work reviews work on breath methane detection for medical investigations.

2. Physiology and disease

Methane gas itself may slow small intestinal transit [6]. There is evidence of slower transit times in methane producers, e.g. in one study, a mean of 84.6 h as opposed to 48.6 h in non-producers. However, this does not mean there is a cause and effect link, simply an association. A recent study [14] showed that administration of the non-absorbable antibiotic rifaximin to a patient with slow transit constipation associated with high methane production both in the fasting state and after ingestion of glucose reduced breath methane levels and improved the constipation symptoms. It has also been shown that intraluminal infusion of methane into the canine small intestine slows transit time (intestinal motility) by up to 59% [15]. The conclusion of the study which involved other animal models and human studies was that methane slows small intestinal transit. The means by which bacteriologically produced
methylene gas slows transit remains unknown; however, research in pulmonary circulation suggests that methane has an effect on smooth muscle through a serotoninergic mechanism [16]. Studies have also shown that after glucose administration, there was a significantly lower serum serotonin concentration in methane producing IBS subjects compared to hydrogen producing IBS patients.

A large number of potentially serious medical conditions have been linked to SIBO including mild/medium depression [4, 17], IBS [10], obesity [18], non-alcoholic steatohepatitis [19], diabetes [20], liver cirrhosis [21], certain cancers [4], rheumatoid arthritis [22] and acne roseacea [23]. The hydrogen breath test has shown utility in establishing a relationship between SIBO and many of these conditions. There is the potential for improved diagnoses and treatment with combined hydrogen/methane breath analyses.

An early study [24] by Haines in 1977 showed 80% of patients with large bowel cancer had detectable breath methane; however, other studies have failed to find this correlation [25]. Several publications have shown an inverse link between inflammatory bowel disease (IBD) and methane production with only small numbers of IBD patients producing methane [26]. The predominant gas of IBD patients was hydrogen with a negligible number being co-producers. More recent work has reaffirmed these observations with methane excretion being stated as clearly associated with alterations in intestinal motility, particularly favoring those with constipation with mean methane excretion higher in subjects suffering from constipation [27].

Methane production has also been found to be more common in other conditions such as diverticulitis and constipation-dominant irritable bowel syndrome (IBS). This former condition is in agreement with higher methanogen concentrations in diverticulosis than in healthy controls, although the differences are not sufficient for an unambiguous diagnosis. One reason given for this is that the diverticula, which are small pouches, may provide a protective niche environment for the growth of methanogens [28]. For the latter condition breath testing to aid in the diagnosis of SIBO may provide a framework for understanding irritable bowel syndrome (IBS) patients. The type of gas produced by bacteria in the gut may be an important factor. Recent work has demonstrated that among IBS subjects, methane is associated with constipation [29] and the degree of methane production with breath testing appears to be related to the degree of constipation. Therefore, methane testing may be used to identify candidates for antibiotic treatment of constipation for immediate and long term alleviation of IBS symptoms [29]. Patients with Crohn's disease, ulcerative colitis and pneumatosis intestinalis have also been reported to have lower levels of methane excretion, 13%, 15% and 11%, respectively [30].

SIBO is frequent in cystic fibrosis; however, diagnosis could very well be underreported as most clinicians use the simple hydrogen breath test and it has been reported that methane is far more frequently detected in cystic fibrosis patients than in other patients. Dual measurement of hydrogen and methane has been recently strongly recommended for cystic fibrosis sufferers [31].

A recent report has linked for the first time higher concentrations of methane detected in breath associated with obese subjects, supporting links between the role of gut flora in obesity [32]. Another study found an increased number of methanogenic bacteria in patients with anorexia [33].

Lactose intolerance has been recognized medically for over a century. The hydrogen breath test [34] in combination with lactose ingestion is widely used as a test method for lactose intolerance (or now normally referred to as malabsorption). However, the hydrogen breath test isn't always sufficient for diagnoses as lactose malabsorbers can give a negative hydrogen breath test. In one study [35], in 11 out of 32 (34%) of lactose-intolerant patients with a negative hydrogen breath test, the methane percentage increase after a lactose challenge was greater than 100%. In the same study, out of 13 subjects having a false-negative breath hydrogen response to lactulose, 11 subjects had a methane percentage increase greater than 100%. Their conclusion was that breath methane measurements might enhance the hydrogen breath test for detecting carbohydrate malabsorption.

Although many people are aware of the condition and avoid dairy products, perhaps what is not so widely known is that lactose is added to many processed foods and drinks and sufferers are still affected [36]. It is now considered that the hydrogen breath test for lactose malabsorption would be better using a combined breath test for hydrogen and methane [36].

Colic is a condition where apparently healthy babies have extended bouts of crying/moaning and occurs in about 10% of babies <3 months old. Malabsorption of lactose in milk is considered to be one of the factors responsible [37]. In the first few weeks of life, infants can have physiological or functional lactase insufficiency that limits absorption of large amounts of lactose. This results in lactose reaching the colon, where it ferments to yield lactic acid, short-chain fatty acids, methane, carbon dioxide and hydrogen. In some cases such fermentation gives rise to excess gas which causes the development of colic. Several studies have related excess crying with excess intestinal gas [38, 39]. Infants that produced higher methane levels at 3 and 6 months of age had significantly (p <0.05) less infantile colic in the first months of life [37] while interestingly the base line hydrogen levels were considerably higher in babies with colic (with little difference between breast fed and formula milk). One can speculate that babies who can readily convert hydrogen to methane could have much less gas in their gut, as the conversion of carbon dioxide to methane uses up four molecules of hydrogen and one molecule of carbon dioxide to produce just one methane molecule.

As stated earlier, methane appears to slow intestinal transit [40] and constipation appears more common among methane-positive patients [41] with the degree of breath methane production correlating with the severity of the constipation [41]. Some caution is needed in the methane test using e.g. lactulose, as complex carbohydrates digested a long time before a lactulose methane test could interfere and give a false positive [42]. Also a single breath test might miss an average 18% of methane producers and sampling over a time range is a superior method [43] and this publication also considered that ethnic differences should be considered in methane breath tests.
3. Methanogenic micro-organisms

The methanogens are obligate anaerobes, primitive microorganisms that are not bacteria but taxonomically belong to the domain Archaea and the kingdom Euryarchaeota. There are two major methanogenic species, Methanobrevibacter smithii [44] and Methanospirillum stadtmaniae [45] that have been isolated from the human intestine. The predominant methane producing organism in humans is M. smithii. In the past, it has been considered that these were the only two Archaea methanogens in the gut; however, recent DNA analyses has shown other species [46]. Other microorganisms in the human gut are also capable of producing methane, such as certain Clostridium and Bacteroides species. The methanogens have a restricted metabolism in which they must reduce simple substrates to methane in order to produce cellular energy. These organisms carry out intestinal hydrogen gas disposal by producing methane, and compete for this substrate with sulfate-reducing bacteria, which generate hydrogen sulfide. Breath methane tests and culture-based methods have traditionally been used to characterize methanogen populations. However, it has been stated that breath tests lack sensitivity as methane is not produced (or detected) in the breath until the methanogens reach a density of about $10^8$ methanogenic bacteria g$^{-1}$ stool [47]. This coupled with the fact that the slow growing organisms are extremely difficult to culture has limited their study. Methanogens have been particularly studied in ruminant species such as cattle, sheep and goats as ruminants can produce 250–500 l of methane per day and production of methane can account for a loss of approximately 6% of the total energy intake of cattle. In humans, methanogenic individuals host methanogens which range from $10^7$ to $10^{10}$ per gram dry weight of feces. A comparative study [48] of methane in the breath from humans in 2006 and 1972 showed that 36.4% of participants were methane producers in 2006, with a mean methane concentration in these producers of 16.6 ppm, which was strikingly similar to the values of 33.6% and 15.2 ppm observed 35 years ago, and neither sex nor age showed a statistically significant relationship to methane production [48].

4. Mechanism of methane production

Methane is considered to be majorly produced in the gut by hydrogenation of carbon dioxide by methanogens, utilizing hydrogen which is also biosynthesized in the gut. Carbon dioxide is also readily available in the gut as it is extensively synthesized by gut bacteria. In culture, methane producing bacteria are capable of producing methane only in the presence of hydrogen, thus proving this biosynthetic route. As hydrogen is used for methane production, when methane is seen using the breath test there is often little or no hydrogen detectable on the test; however, coproduction is readily detectable when it does occur with modern hydrogen/methane detection systems. It is now considered that methanogens are mainly responsible for methane production; however, a study in 1985 reported that it was still not clear whether methane in the gut arose from relatively few methanogens or a large number of gut organism such as Bacteroides and Clostridia [30]. Substrates other than carbon dioxide can be used to biosynthesize methane [49] e.g. methanol, formate and acetate; for instance, one notable gut bacteria Methanosphaera stadtmaniae can produce methane from methanol in the gut [49].

5. Methane and humans

It is considered that about 80% of the methane is excreted by flatus and about 20% in breath. Methane is not utilized by humans, so it is excreted either as flatus, or it traverses the intestinal mucosa and is absorbed into the systemic circulation and excreted unchanged through the lungs. It has been stated [25] that methane has not been reported to be detected in children until 3 years of age, and then methane production increases until the adult distribution is reached. Although the latter is true, there have certainly been reports of methane in younger children than this, e.g. analysis of the stool samples revealed that methane was produced at concentrations >2 ppm by 15.3% of the infants at age <3 months and by 46.4% of infants at age >6 months [37]. In a study of pediatric methane production, breath methane was analyzed in a large study of healthy subjects in the Tel-Aviv area [50] and 18% of children aged 14 had detectable breath methane. From age 14 on, the incidence of methane production increased sharply to reach that of the adult population (49.4%). Also notable in this study was that in the adolescent and adult groups, significantly more females than males produced methane. Numerous studies measuring breath methane have been conducted, and it is estimated that approximately 30–62% of healthy adults excrete methane [9, 30]. Traditionally, adults have been classified as methane producers versus non-producers based on breath methane status. However, patients who do not excrete methane in the breath can in fact have methane present in colonic gas [30]. This has been corroborated through fecal incubation studies demonstrating that the majority of individuals can produce methane in the colon. The investigators estimated that approximately $10^8$ methanogenic organisms per gram dry weight of stool are needed to generate enough methane to be detected by breath analysis [47]. These studies suggest that a higher percentage of individuals than previously thought may produce methane, but only when a certain threshold is reached will methane be detectable in the breath. In summary, although methane is produced in amounts measurable on the breath in up to 50% of the population, it would appear that the non-methane producers are mostly not lacking methanogenic bacteria; however, there are unknown factors controlling the numbers of methanogens or alternatively the amount of methane they produce. There is a theory that sulfate-reducing bacteria are in competition with methanogens for hydrogen in the colon and this reduces methane production. This now appears to be an unlikely reason; in fact, methanogenic organisms outcompeted other hydrogen-consuming bacteria for hydrogen [51].

Methane production in the human colon shows significant inter-ethnic differences. An African study [52] showed that methane producers were rural blacks (84%), urban blacks (72%), white (52%) and Indian (41%). Another study [43]
presented data with some similarities: Caucasians (48%), blacks (45%), Indians (32%) and orientals (24%).

The stomach is normally considered to be a harsh environment for bacteria; thus, very low levels of hydrogen and methane would be expected to be produced there. These gases were measured endoscopically for the first time [53], in 2006, the authors’ aim being to discover a new method for bacterial overgrowth detection in the stomach. By assessing hydrogen and methane levels it was surprisingly determined that 15% of patients (473 in total) were considered to have intragastric fermentation even after overnight fasting (regardless of abdominal symptoms). No correlation was found with *H. pylori* the most commonly tested for stomach bacterium (due to its health implications). Previous gastric surgery appears to influence the production of methane in the stomach probably by affecting the growth of methane producing bacteria. It is well known that the number of microbial flora present in the stomach is affected by gastric pH, and increases with higher pH, and this may affect gas production. Fermentation in the stomach or proximal small intestine is medically interesting as it can inhibit gastric and pancreatic secretions, and also influence lower esophageal sphincter function in gastrosophageal reflux disease [54].

The location of bacterial overgrowth might be determined by breath sampling; however, very little work appears to have been undertaken on stomach hydrogen and methane generation and its diagnostic potential.

6. Analytical methods for methane detection and breath testing

There are a range of methods for the detection of methane from conventional gas chromatography methods to spectroscopic techniques developed for atmospheric monitoring. Some of these techniques have been or could be applied to monitoring methane levels in breath. A previous review of analytical methods for the detection of methane has been published [55]. For breath methane detection, methods are needed which can measure methane from about 1–100 ppm. There is little point in systems being more sensitive due to the background atmospheric methane which is about 1.7 ppm.

Methane can be detected via gas chromatography using a variety of detectors. These include flame ionization detectors [56–59]Author Vi, thermal conductivity detectors [60], pulsed helium discharge ionization detectors [61] and mass spectrometry [62]. Breath methane has also been measured in humid atmospheres using selective ion flow transfer mass spectrometry (SIFT-MS) [63].

A number of simple sensors such as those based on metal oxides [64]Author V, catalytic based sensors such as pellistors [65] or other catalytic sensors [66], semistors [67] and piezoelectric sensors [68, 69] and SAW devices [70] have been deployed in the detection of methane. New materials such as carbon nanotubes have been utilized to enable room temperature detection of methane [71]. In addition to these solid-state sensors, a number of electrochemical sensors have been used to detect methane; these include amperometric sensors [72] and methane biosensors which utilize methanotrophic bacteria [73, 74] and also methane fuel cells [75].

There are a number of spectroscopic based methods for detecting methane. These are typically based on mid- or near-infrared diode laser spectroscopy [76]. A recent review of near-infrared methane detection methods based on tunable diode lasers with comparison of detection limits has been published [77]. Many methane detectors are designed for environmental monitoring or as leak detectors where low limits of detection are not required. There are relatively few reports of methane detectors used for breath analysis in humans or animals. As mentioned, SIFT-MS has been utilized to measure methane on the breath of human subjects. Fourier transform infrared has been utilized for the detection of breath methane in dairy cows [78]. Continuous wave optical parametric oscillators in combination with photoacoustic spectroscopy have shown utility in the measurement of breath methane in human subjects [79]. A pulsed discharge helium ionization detector (PHID) coupled to gas chromatography has been used for the simultaneous measurement of hydrogen and methane on breath [80]. It is quoted as having a better sensitivity than a TCD for hydrogen or FID for methane which are typically used as detectors in this analysis. The quoted limit of the detection for the PHID was 0.3 ppmv versus quoted values of 6 ppm for both hydrogen and methane when injecting 1 ml of gas onto an 8 m packed column [79].

A photoacoustic method has been used for the detection of methane emanating from the human skin. The detection limit for the device was found to be 0.25 ppm with an integration time of 12 s which was adequate for detecting methane from the skin [81].

There are a number of companies selling methane detectors ranging from inexpensive solid-state devices such as metal oxide based [82] and catalytic pellistors [83] to more expensive optical detection systems. Also available are portable flame ionization detectors.

There are commercial instruments available which are capable of the simultaneous measurement of breath methane and hydrogen specifically designed for monitoring carbohydrate malabsorption syndromes or SIBO. The Quintron BreathTracker™ SC [84] separates the components by the basic principle of gas chromatography, using room air as the carrier gas, which is pumped through the system by an internal circulating pump. Hydrogen and methane are separated from all other reducing gases and from each other, and are carried past a solid-state sensor. The sensors are reported to be affected only by reducing gases, so it is unaffected by other gases in the sample; it can also employ a carbon dioxide correction factor.

The Lactotest 202 instrument [85] uses an electrochemical hydrogen sensor and an infrared sensor for methane and carbon dioxide detection. There is also the GastroCH4ECK™ instrument [86] which simultaneously measures hydrogen, methane and oxygen in patient’s breath by using an infrared sensor for measuring methane and hydrogen levels by measuring with an electrochemical sensor. It has been stated that [86] unlike other commercially available devices the GastroCH4ECK™ allows users to collect direct breath samples as well as samples from a breath bag.
There are also a number of companies which offer a breath analysis service which incorporates methane detection. Most of these commercial tests center around the detection of carbohydrate malabsorption syndromes. Patients provide samples which are sent to the companies in appropriate containers and can be measured via gas liquid chromatography, or by the more bespoke methodology described above.

7. Animals and breath methane measurements

Breath tests for animals potentially offer a rapid, noninvasive diagnostic test methodology. In animals digestion of ‘rougheage’ causes methane production and ruminant animals appear to have evolved to cope with methane production. As in humans methanogens are the key bacteria producing methane. Methanogens are a small proportion of the total rumen microbial population. Interestingly reducing the numbers of methanogens in the rumen can reduce methane production, apparently without detriment to the digestion process. A vaccine that acts against the methanogens reduces methane by up to 7.7%, and antibiotics (such as rumensin) added to the diet of ruminants can reduce methane production. Change in diet for instance in dietary fat definitely leads to methane reduction. The main reason for methane analyses in the animal world is for research purposes to reduce animal emissions, in attempts to reduce global warming [87]. Production of methane accounts for a loss of approximately 6% of the total energy intake of cattle. Cows grazing pasture can produce up to 350 l of methane a day compared to those fed grain where production drops to 100 l a day [88]. This is attributed to the faster transit times of more easily digested food therefore reducing the excretion of methane via the mouth, the dominant excretion pathway for ruminants.

Precision studies of methane production in cattle employ a whole animal respiration chamber [89]. The limitation is that it can take many hours to study just one animal. The reason for studying methane production in herds of cattle is to selectively breed cattle with lower methane emissions to lower the environmental impact of dairy farming. A simpler method taking only 1 h was deployed to measure methane production in sheep [90] and more recently an FTIR method has been used for methane from cows [91]. Noninvasive methods applicable to commercial herds have utilized hand-held laser reflectance instruments [92].

SIBO can also affect dogs [93] and they usually present with poor physical condition and have chronic diarrhea and loose stools, along with flatulence. Weight loss is common. Vomiting may or may not be associated. Blood tests can help diagnose SIBO; however, a non-invasive hydrogen breath test for the detection of SIBO in dogs has been established [93]. A positive result is very suggestive of SIBO, negating the need to culture duodenal juice. However, a negative test does not rule out SIBO, and in these cases culture of duodenal juice is stated to be indicated [93]. However, it could very well be that a detection system using hydrogen supplemented by a methane test will increase the effectiveness of breath tests for animals [94].

8. Conclusion

Breath tests can be valuable in helping to evaluate functional bloating, diarrhea, constipation and suspected malabsorption syndromes. A combined hydrogen and methane breath test has been shown to be superior for the diagnosis of carbohydrate malabsorption syndromes and SIBO. These tests are simple and safe alternatives compared to more invasive procedures such as biopsies and/or obtaining aspirates for culturing.

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