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EFFECTS OF PREBIOTIC INTAKE ON GLYCEMIA AND SERUM CHOLESTEROL CONCENTRATION IN OBESE AND EUTROPHIC DOGS: A SYSTEMATIC REVIEW

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Abstract
Sedentary behavior and low physical stimulation in companion animals have increased obesity and related metabolic disorders. Dietary fibers, such as prebiotic polysaccharides, are potential adjunct therapies for these conditions. This systematic review evaluated the effects of prebiotic intake on blood glucose and serum cholesterol levels in dogs. Using the PICOS strategy, we focused on eutrophic and obese dogs treated with prebiotics (Intervention) versus placebo diets (Comparison) in randomized clinical trials (Study design). The outcomes assessed were blood glucose and serum cholesterol concentrations. Two reviewers independently searched five databases and grey literature, assessing bias with the SYRCLE protocol. From 2,518 articles, 22 were pre-selected for full-text review, and nine met the inclusion criteria. Qualitative analysis revealed that prebiotic intake significantly reduced serum cholesterol in 57% of studies, particularly in non-obese dogs (75%), without exceeding physiological limits. However, blood glucose levels were unaffected in most studies (71%). The studies demonstrated low risk of bias, though blinding procedures were often poorly described. Due to study heterogeneity, meta-analysis was not feasible. We concluded that prebiotic ingestion effectively reduces serum cholesterol in dogs, with minimal impact on blood glucose levels.

Keywords: Animal Nutrition. Prebiotics. Systematic Reviews as Topic.

1. Introduction

Recently, people's interest in owning pets has surged, and the global pet population now exceeds 1.6 billion. The United States ranks first in the number of domestic animals, followed by Brazil and China. Among these pets, approximately 700 million are dogs worldwide (Smith et al. 2019).

The strong bond between humans and animals enhances pet owners' quality of life and offers physical and mental health benefits. Living with pets reduces stress levels and blood pressure, helping to prevent heart disease and treat depression (Dotson & Hyatt 2008; Levine et al. 2013). However, while pet ownership benefits humans, this close relationship can adversely affect animals. Pets often adopt their owners' lifestyle habits, leading to various health problems. For example, lack of stimulation, stress from urban noise, and frequent or inadequate transportation directly impact animal health (Beerda et al. 1997). A notable negative outcome is the increased incidence of obesity in dogs; they live in confined spaces and receive highly caloric and palatable foods from their owners (German 2006).

To address these health concerns, pet owners and the industry are increasingly interested in developing alternative diets with prebiotic supplements that boost animals' intestinal and immune health (Finet et al. 2021). Prebiotics in the diet act as substrates for beneficial gastrointestinal microorganisms, modulating the intestinal microbiota and improving overall health (Delzenne & Cani 2005). Among the main prebiotics, dietary fibers stand out; the body does not digest or absorb them. Pet food manufacturers use various types of dietary fiber from different sources. In canine diets, the most common prebiotics are inulin, fructooligosaccharides (FOS), oligofructose (OF), and mannan oligosaccharides (MOS) (Swanson et al. 2002).

Despite the increasing use of prebiotics in pet nutrition, scientific evidence of their clinical effectiveness remains scarce and controversial (Robertson 2007; Tolhurst et al. 2012). Therefore, we aimed to systematically evaluate the results of randomized clinical trials—both parallel and crossover designs—involving prebiotic consumption by dogs to determine their effectiveness in controlling glycemic and serum cholesterol levels.

2. Material and Methods

This systematic review adhered to the PRISMA 2020 guidelines (Page et al. 2021).

Eligibility Criteria

Central Question

Employing the PICOS framework (Population, Intervention, Comparison, Outcome, Study Design), the central research question was formulated: Does prebiotic ingestion by obese and/or eutrophic dogs modulate glycemia and serum cholesterol levels compared to placebo? Figure 1 depicts the methodological schema for this inquiry.

Question

Can the intake of prebiotics by obese and/or eutrophic dogs modulate glycemia and serum cholesterol concentrations compared to a placebo treatment?

PICOS	
Participants	Obese and/or eutrophic dogs.
Intervention or exposition	Use of prebiotics.
Comparison or control	Placebo.
Outcome measure(s)	Glycemia and serum cholesterol concentrations.
Study Design	Randomized clinical trial (parallel or crossover).

Figure 1. Diagram illustrating the formulation of the central research question using the PICOS framework, detailing Population (P), Intervention (I), Comparison (C), Outcome (O), and Study Design (S).

Inclusion Criteria

Only studies assessing both obese and eutrophic dogs that received prebiotics (intervention) and were compared to placebo-fed counterparts (comparison) were included. These studies, which examined changes in glycemia and serum cholesterol levels, adhered to randomized clinical trial protocols (parallel or crossover).

Exclusion Criteria

Studies of dogs with chronic inflammatory diseases, diabetes, or in the gestational period were excluded, along with those involving concurrent non-prebiotic therapies.

Search Strategy

Comprehensive literature searches were conducted across PubMed (https://pubmed.ncbi.nlm.nih.gov/), EMBASE (https://embase.com/), Web of Science (http://isiknowledge.com/), Scopus (https://www.scopus.com/), and Cochrane (https://www.cochranelibrary.com/), supplemented by gray literature reviews via Google Scholar (https://scholar.google.com.br/) and Opengrey (https://opengrey.eu/), culminating in July 2022. The searches, which included keywords and MeSH terms like "dog," "prebiotic," "glucose," and "cholesterol," utilized database-specific filters without imposing language or publication date limitations. Retrieved studies were catalogued using Rayyan software (https://www.rayyan.ai/) and the Mendeley reference manager (https://www.mendeley.com/).

Analysis of Studies

Initial screening involved two independent reviewers (TCL and FOFM) who analyzed titles and abstracts against the eligibility criteria, excising duplicates via Rayyan. A secondary, detailed examination of the pre-selected studies' full texts was then conducted. Studies aligning with the PICOS framework were retained; discrepancies were mediated by a consensus session with senior supervision (LJP).

Data Extraction

Data were extracted and catalogued, encompassing references, experimental design, sample size, demographic details (age, sex), body condition (obese or eutrophic), prebiotic type, analytical methodologies, trial duration, statistical models, and outcomes regarding glucose and cholesterol levels relative to controls.

Risk of Bias

The risk of bias was assessed using the Risk of Bias (RoB) tool from SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) (Hooijmans et al. 2014), which is endorsed for systematic reviews that involve animal studies. SYRCLE's protocol evaluates ten types of biases, namely selection bias (concerning the generation of allocation sequence, baseline characteristics among animals, and allocation concealment), performance bias (related to the randomization of allocation and blinding of those administering treatments), detection bias (involving the selection and blinding of researchers who assess the data), attrition bias (determining whether results were complete), reporting bias (examining if the results were reported selectively or with bias), and other potential biases identified by the researchers.

The representation of this tool is a table, where the presence of a positive sign (+) indicates that a criterion was met without associated bias, whereas a negative sign (-) suggests that a parameter was either improperly applied or not reported, thereby increasing the risk of bias. In accordance with the SYRCLE protocol, two researchers, TCL and FOFM, independently conducted the bias risk analysis. Subsequent to their independent evaluations, a consensus session was convened to reconcile any differences.

3. Results

Study Selection

The database searches yielded 2,518 studies. We removed 196 duplicates, leaving 2,322 studies for evaluation based on their titles and abstracts. We selected 22 potential articles for full-text review. Of these, we excluded 13 articles: 11 did not evaluate glycemia or lipid profiles, one focused solely on pregnant dogs, and one involved other simultaneous therapies. Consequently, only nine studies met the eligibility criteria. Figure 2 illustrates the research selection process according to PRISMA (Page et al. 2021).

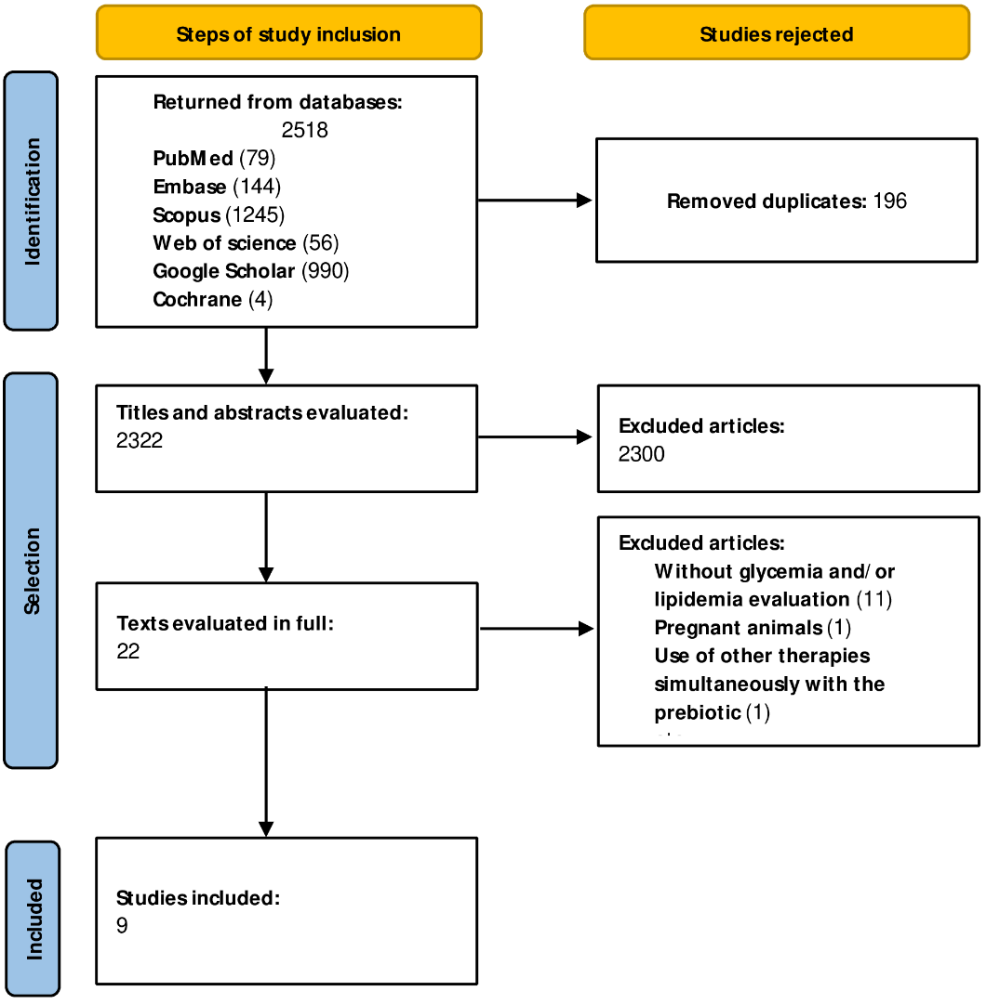

Figure 2. Article Search and Selection Flowchart.

Characteristics of the Studies

Table 1 presents the characteristics of the studies included. This study incorporated nine articles based on established criteria. Five articles assessed eutrophic animals, and four examined obese dogs. Experiment durations ranged from 14 to 259 days, with participant counts varying from 5 to 14 animals.

Most studies (78%) focused on evaluating Beagle dogs. Among eutrophic animals, 67% consisted of Beagles and 33% of Spitz. All studies involving obese animals exclusively utilized Beagle dogs (100%). Uncastrated females comprised 25% of the animals, followed by castrated females (16%), castrated males (13%), and uncastrated males (10%). Three studies, encompassing 36% of the total dogs, did not specify the sex of the animals.

FOS emerged as the main fiber used in the studies (45%), applied at levels of 1%, 3%, and at 5% when combined with beet fiber (4:1). MOS ranked as the second most utilized fiber source (22%), at levels of 1% and 1.5%. One study (11%) incorporated inulin-type fructans at an inclusion rate of 0.5%. Another study (11%) employed a mixture of FOS, MOS, inulin, beet pulp, and cellulose at a 5% inclusion level. The last study (11%) utilized beta-glucan at a level of 1%.

Table 1. Data from Included Studies

Reference	Sample	Body condition	Intervention	Duration	Statistical analysis	Effect on glycemic levels	Effect on lipidic profile
Eutrophic animals (non-obese)							
Diez et al. (1997)	8 adult Beagle dogs (male)	Non-obese	A: Control, without additional fiber B: 5% mixture of fructo-oligosaccharides and beet fiber (4:1) C: 10% mixture of fructo-oligosaccharides and beet fiber (4:1)	182 days	ANOVA	Significantly lower at 5% and 10% concentrations.	Significantly lower at 5% and 10% concentrations
Kore et al. (2012)	5 adult Spitz dogs (sex not specified)	Non-obese	Control: Isolated basal diet MOS: Mannan-oligosaccharides at 1%	28 days	ANOVA	No significant differences	Decrease in plasma triglycerides
De Souza Nogueira et al. (2019)	8 adult Beagle dogs (female)	Non-obese	CT: 5% cellulose FP: 5% fiber and prebiotic blend (cellulose, beet pulp, inulin, MOS, and FOS SE: 0.02% saccharin and Eugenol (phytomolecule) FSE: 5% fiber and prebiotic blend + 0.02% saccharin and eugenol	56 days	Mixed models	No significant differences	No significant differences
Pawar et al. (2017)	10 Spitz pups (sex not specified)	Non-obese	Control: no supplementation MOS: supplemented with MOS (derived from the cell wall of *Saccharomyces cerevisiae* at 1.5% of the diet)	150 days	ANOVA	Not evaluated	Reduced total cholesterol levels
Ferreira et al. (2018)	14 adult Beagle dogs (sex not specified)	Non-obese	Control: no supplementation Supplemented: Beta-glucan at 1%	71 days	ANOVA	No significant differences	Reduced total cholesterol, LDL-c and VLDL-c levels

Table 1. Continued.

Obese animals							
Alexander et al. (2018)	9 adult Beagle dogs (female)	Chronic obese	Placebo: cellulose, 0.25% of the diet Low dose: inulin-type fructans (Orafti® SIPX) 0.5% of the diet High dose: inulin-type fructans (Orafti® SIPX) 1% of the diet	14 days	Mixed models	No significant differences	Not evaluated
Apper et al. (2020)	6 adult Beagle dogs (neutered females)	Obese (overweight for 12 months before the experiment)	Control: No Supplementation OF: 1% DM oligofructose (OF, Orafti® OLF, Beneo, Oreye, Belgium) ScFOS: 1% DM scFOS (scFOS: degree of polymerization, 3–5, Profeed®, Tereos, Marckolsheim, France)	259 days	ANOVA	No significant differences	No significant differences
Respondek et al. (2008)	8 adult Beagle dogs (4 neutered males, 4 neutered females)	Obeses (induced by diet)	Control: no supplementation scFOS: scFOS (degree of polymerization, 3-5, Profeed, Beghin-Meiji) (1% dry matter over feed)	105 days (after 98 days of obesity induction)	Mixed models	No significant differences	No significant differences
Ricci et al. (2011)	12 adult Beagle dogs (6 neutered males, 3 unneutered females, 3 neutered females)	Chronic obese	Control: commercial high-protein diet with caloric restriction Test: Same diet as control group, enriched with sc-FOS to reach 3% DM	224 days	ANOVA	Significantly lower at 3% concentration	Significantly lower at 3% concentration

Studies involving obese dogs employed only two fiber sources (FOS and inulin-type fructans). Among these studies, 75% utilized FOS at a 1% level, while the remaining 25% incorporated inulin at 0.5% and 1% levels. In studies with non-obese animals, MOS emerged as the most frequently utilized fiber source (40%), at inclusion levels of 1.5% and 1%. FOS at 1% appeared in 20% of these studies. A mixture of various fiber sources also featured in 20% of the studies at a 5% inclusion level. Beta-glucan appeared in the remaining 20% of the studies at a 1% level.

Qualitative Data Analysis

Among all evaluated studies, most (78%) reported no significant influence of fibers on blood glucose levels. However, fibers significantly reduced cholesterol concentrations in 50% of the studies. When we separated the studies based on animal weight, we observed diminished effects in overweight animals: only 25% of studies reported a significant reduction in blood glucose and 33% in cholesterol. In contrast, studies with eutrophic animals showed beneficial effects in 25% regarding blood glucose levels, 20% regarding plasma triglyceride levels, and 60% regarding serum cholesterol concentrations. Notably, one study did not evaluate blood glucose levels, and another did not evaluate serum cholesterol concentrations (Pawar et al. 2017; Alexander et al. 2018).

Risk of Bias

Table 2 presents the results from using the SYRCLE tool. We assessed several criteria: sequence generation, baseline characteristics, random housing, random outcome assessment, incomplete outcome data, and selective outcome reporting, all of which were satisfactory across the studies with no associated bias (100%). However, only one of the evaluated studies (11%) reported blinding of participants and personnel. Additionally, none of the studies offered clear information on allocation concealment or blinding of outcome assessment.

Table 2. Risk of bias outcomes.

Reference	1	2	3	4	5	6	7	8	9	10
Obese animals										
Diez et al. (1997)	+	+	-	+	-	+	-	+	+	+
Ferreira et al. (2018)	+	+	-	+	_	+	-	+	+	+
Kore et al. (2012)	+	+	-	+	-	+	-	+	+	+
De Souza Nogueira et al.(2019)	+	+	-	+	-	+	-	+	+	+
Pawar et al. (2017)	+	+	-	+	-	+	-	+	+	+
Eutophic animals										
Alexander et al. (2018)	+	+	-	+	+	+	-	+	+	+
Apper et al. (2020)	+	+	-	+	-	+	-	+	+	+
Respondek et al. (2008)	+	+	-	+	-	+	-	+	+	+
Ricci et al. (2011)	+	+	-	+	-	+	-	+	+	+

1) Sequence generation. (2) Baseline characteristics. (3) Allocation concealment. (4) Random housing. (5) Blinding of participants and personnel. (6) Random outcome assessment. (7) Blinding of outcome assessment. (8) Incomplete outcome data. (9) Selective outcome reporting. (10) Other sources of bias.

4. Discussion

Interest in using prebiotics in pets has surged due to previous studies demonstrating positive effects of fiber consumption on blood sugar and lipid profiles in various animal models and humans

(Busserolles et al. 2003; Giacco et al. 2004; Clark et al. 2006). Our study revealed significant effects of prebiotic consumption on serum cholesterol concentration in dogs, more pronounced in eutrophic animals but still within normal ranges. However, the effects on blood sugar levels appear less evident for both eutrophic and obese animals.

We identified eight studies meeting the eligibility criteria, with four addressing non-obese dogs and four focusing on obese animals. As previously observed in systematic reviews, the included studies exhibited considerable heterogeneity, using four different types of dietary fiber with prebiotic effects (Le Bourgot et al. 2018; Canaan et al. 2021). The dosages applied in the experiments also varied, with eight different inclusion levels tested. Furthermore, the number of participants ranged widely, from 5 to 12 animals, as did the duration of the tests, which spanned from 14 to 259 days. Thus, the lack of a defined protocol among the studies complicates comparisons and makes defining the certainty of evidence difficult. Due to the high heterogeneity, we could not perform a meta-analysis.

The significant reductions in lipid profiles observed in some studies align with results obtained in rodents receiving 10% prebiotic inclusion (Delzenne and Kok 1999; Busserolles et al. 2003) and in humans consuming doses of 10 to 20 g/day (Causey et al. 2000; Giacco et al. 2004). This consistency suggests that the intestinal microbiota regulates cholesterol metabolism by generating fermentative by-products in the colon, mainly short-chain fatty acids (SCFAs) (Bäckhed et al. 2004; 2007). A probable mechanism involves bile acid circulation, the main route of cholesterol excretion. For instance, in mice, inulin reduced bile acid solubility, leading to increased fecal excretion (Remesy et al. 1993). Additionally, increased SCFA production decreases luminal pH in the small intestine, hindering bile acid reabsorption. SCFA production also benefits the host by limiting pathogen growth through reduced luminal pH and by providing energy to colonocytes (Roediger 1982; Topping and Clifton 2001).

However, studies suggest that prebiotics may not efficiently reduce triglycerides in advanced stages of hypertriglyceridemia, possibly due to decreased bile acid excretion in these animals' feces. This phenomenon may explain the results found in obese dogs (Vanhoof and De Schrijver 1995; Respondek et al. 2008). Various factors can reduce bile acid excretion in the feces of dogs with hypertriglyceridemia. Liver dysfunction associated with hypertriglyceridemia may impair bile acid synthesis or secretion, decreasing their intestinal output. Disruptions in normal enterohepatic circulation can also limit bile acid availability for excretion. Furthermore, hyperlipidemia may induce biliary stasis or gallbladder dysfunction, reducing bile flow into the intestines. Dietary factors, such as high-fat intake, can alter lipid metabolism and impact bile acid absorption, potentially causing malabsorption issues. Finally, changes in gut microbiota can interfere with the conversion of primary to secondary bile acids, further affecting fecal bile acid levels (Kakimoto et al. 2017; Teixeira et al. 2020; Vecchiato et al. 2023).

Although studies in humans indicate that dietary fiber intake improves postprandial glycemic index (Clark et al. 2006; Mollard et al. 2014), we did not observe such improvements in most of the articles included in our study for both obese and eutrophic dogs. Our observations, based on evaluations of fasting glucose concentrations in non-diabetic animals, suggest that fasting glucose may not serve as a sensitive marker for assessing the effects of prebiotic supplementation on glucose homeostasis, especially given the scarcity of studies with diabetic dogs (Apper et al. 2020). Consistent with our findings, similar studies in other animal models (Verbrugghe et al. 2009; Le Bourgot et al. 2018) and in humans (Kellow, Coughlan, Reid 2014) also failed to find significant effects of prebiotic intake on fasting blood glucose levels. Moreover, the level of prebiotic inclusion may influence outcomes, as low dosages might be insufficient to elicit significant effects. Our previous studies have demonstrated a dose-dependent effect of fiber intake (de Sales Guilarducci et al. 2020; Azzi et al. 2021). Therefore, we need further research to determine the appropriate inclusion levels for dogs.

Our evaluation of bias risk revealed low overall risk, although the main flaws involved the absence or non-description of allocation concealment and blinding of outcome assessment. Most authors did not provide clear information in this regard. In animal trials, researchers often overlook blinding criteria (Kilkenny et al. 2009; Cochrane 1972). However, all included studies met 70% of the evaluated criteria, demonstrating good methodological quality.

5. Conclusions

Dietary fibers used as prebiotics effectively reduce plasma cholesterol in non-obese dogs but do not impact glycemia in obese dogs.

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Ethics Approval: Not applicable.

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