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ROLE OF GUT MICROBIOTA IN ANIMAL HEALTH

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ABSTRACT

It is widely believed that the large number of different micro-organisms present in the gut would be beneficial to the host in a number of ways, including nutritional, digestive and immunological functions, rate of growth and resistance to diseases. This study aims to determine the diversity of gut flora present, relate this to different parameters of normal physiology and disease, and see if it could be advantageously altered, either by dietary means or by supplementing with 'desirable' micro-organisms. As all features of interest could be quantified, an essentially descriptive design was considered most suitable on 150 animals selected by stratified random sampling. Most information would be derived from examination of faeces, clinical and other measurements, together with available literature and other information sources. Micro-organisms would be harvested from suitable samples for DNA sequence analysis (16S rRNA) to assess the degree of diversity. A range of statistics, including correlations, multiple regression, chi-squared and ANOVA, would be calculated. Many parameters would be expected to be significantly correlated with the degree of diversity, including several measures of nutritional state, growth rate and immunity. It would be anticipated that animals with one or more diseases would have a lower diversity, and that supplementation with desirable organisms may be advantageous in several respects. Provision of good quality nutrients in sufficient quantities, the presence of a great diversity of beneficial organisms in the gut, and sensible

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restriction to where warranted to other uses of antimicrobials, would all seem desirable. Many types of intervention to alter gut flora might be expected to result in improved general state of health, less disease and less need for antibiotics, all with economic benefits. Similar studies on many other species would seem worthwhile.

Keywords: Gut Microbiota, Animal Health, Gut Microbiome, Immune Regulation, Nutrient Metabolism, Probiotics, Animal Nutrition, Gut Dysbiosis.

1. INTRODUCTION

1.1 Background of the Study

A large and diverse population of microbes resides in the GI tract of many animal species, and is involved in many aspects of normal nutrition, health, immune function and general homeostasis. It seems reasonable to assume that many of these organisms confer some benefit on their host, since they have co-existed with them over many years. For ruminants, bacteria and other micro-organisms are able to ferment plant wall carbohydrates and many other substrates, providing varying amounts of energy in the form of SCFA. Many factors, including diet, environment and over-use of antibiotics, may adversely affect the population of beneficial organisms. It would therefore be highly valuable to know more about the identity of these organisms and their interactions with the host, in order to enhance health, resistance to disease and animal production (Flint et al., 2012).

1.2 Concept and Composition of Gut Microbiota

The gastrointestinal tract contains an extremely wide range of bacteria, archaea, fungi, protozoa and viruses. In general, most animal GI tracts are dominated by members of the Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. There will be major differences in the types and numbers of these organisms between species, and with age, diet, environment and the general state of health. As would be expected, rumen microflora have a large number of organisms able to degrade various plant polysaccharides, while there will be variations amongst monogastrics. Many organisms, for example Lactobacillus spp. and Bifidobacterium spp., have a role in maintaining normal gut function and preventing colonization by potentially pathogenic organisms. A high degree of diversity and stability would be expected to be beneficial to gut function and overall metabolism (Ley et al., 2008).

1.3 Importance of Gut Microbiota in Animal Health

The gastrointestinal microbes aid in digestion of various nutrients, synthesis of vitamins, modulation of host immunity, and provide protection against potential pathogens. Many organisms are able to synthesize bacteriocins and other compounds inhibiting growth of undesirable organisms, as well as compete for available substrates and receptors, all contributing to colonization resistance. Short chain fatty acids, including acetate, propionate and butyrate, are products of fermentation and can be used as an energy source and are important in maintaining the gut as an

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effective barrier. Microbes play a role in immune maturation and can protect against some diseases. Disruption of the normal complement of bacteria in the gut (dysbiosis) may result in a range of inflammatory conditions, metabolic disease and poor growth. It is therefore important to maintain a healthy and stable population of gut bacteria (Kogut and Arsenault, 2016).

1.4 Relationship between Gut Microbiota and Animal Physiology

The numerous host systems are in intimate contact with the gut flora via metabolism, immunity, endocrine and neural mechanisms. Many substrates ingested with the diet can be digested by microbes, with formation of a range of products with various biological effects on the host. Short-chain fatty acids have a number of important effects on the gut epithelium, energy metabolism and immune processes, and in ruminants volatile fatty acids are a very significant source of metabolisable energy. Microbes may be involved in the production of various vitamins and in the metabolism of bile salts, as well as maintaining features of the intestinal barrier. There is also evidence that the gut flora can signal to the brain, altering a range of behaviours and other functions. All of these could be important determinants of rates of growth and feed conversion, responses to challenges, and general state of well-being (Flint et al., 2012; Cryan et al., 2019).

1.5 Statement of the Research Problem

Although there is growing awareness of the importance of the gastrointestinal flora to the health of a wide range of animals, much is unknown about how microbial diversity may relate to feed conversion, general immunity, susceptibility to various conditions and so on. A poor diet, various stresses, disease and excessive and inappropriate use of antibiotics will all alter gut flora populations. Consequences may include various digestive upsets, poorer feed conversion and growth, and selection for antibiotic resistance. There will be differences in population levels and types of organisms between different animal species, so determining general "healthy" markers may be difficult. This work aims to investigate relationships, if any, of the gut flora with the general state of health of animals, and the effects that various diets and probiotics may have.

1.6 Significance and Scope of the Study

This work would be beneficial to increase knowledge of the intestinal microflora in relation to diet, host immune function, rates of growth and resistance to diseases. It covers a wide range of topics, including diversity of different microbes, metabolism of various nutrients, disturbances of normal gut flora and related host responses, and use of various beneficial organisms and feed additives. Sequencing of 16S rRNA genes would allow determination of various bacteria present and correlation with different parameters. The results would be of interest to a range of scientists, including basic and applied animal scientists and producers. Understanding the gut microbiota may allow greater use of alternatives to antibiotics, better welfare and higher levels of production in an environmentally friendly manner (Gresse et al., 2017).

2. REVIEW OF LITERATURE

2.1 Diversity and Distribution of Gut Microbiota in Animals

Ley et al. (2008) studied the association of the intestinal microflora with evolution in a

range of mammals. Both phylogeny and diet were important determinants of the diversity of microorganisms present in the gut. As expected, a wider range and diversity of microorganisms was found in herbivores compared with carnivores. It was suggested that the ability of these microorganisms to utilize various nutrients available depending on diet would be beneficial in digestion and metabolism, and in the evolution of various mammalian orders.

Muegge et al. (2011) all diets appeared to have a major effect on the functions represented by the gut microbes, regardless of phylogenetic relatedness. Different adaptations could be expected, depending on whether the host was a herbivore, omnivore or carnivore. Diet was therefore likely to play a large role in the type of microorganisms present, their various functions, and overall stability of this ecosystem.

2.2 Role of Gut Microbiota in Digestion and Nutrient Absorption

Flint et al. (2012) described various roles for microorganisms in the digestion of nutrients and other metabolic functions. Carbohydrates, fibres and resistant starch can all be fermented by bacteria within the gut to produce a range of short-chain fatty acids, some of which can be used as an energy source by the host, as well as having other beneficial effects on gut function. Thus many important functions relating to nutrient supply can be attributed to gut microbes.

Bäckhed et al. (2004) also showed that gut flora play several roles in affecting energy harvest from various substrates, and in the metabolism of these substances by the host. As a consequence of colonisation with microorganisms there was greater deposition of fat deposits. Thus gut flora have a number of significant effects on aspects of the host's nutrition.

2.3 Gut Microbiota and Immune System Regulation

Hooper et al. (2012) reviewed various aspects of the interaction of the gut flora with the host immune functions. They described a role for many commensals in promoting immune development, maintaining integrity of the gut, and defending against pathogens, with a wide range of signals to the innate and acquired immunity. Importantly, appropriate interactions with microbes were considered required to sustain a state of immune balance and avoid inappropriate inflammation.

Belkaid and Hand (2014) reviewed current knowledge of the effects of microbiota on various aspects of immunity and inflammation. A wide range of positive effects on immune cell maturation, Treg function and general host defenses have been described. Altered microbial communities could therefore lead to various immune dysregulation and inflammation. It is important that a balanced flora is maintained to allow tolerance and effective protection from pathogens.

2.4 Role of Microbial Metabolites in Animal Health

Koh et al. (2016) reviewed the roles of short chain fatty acids generated by bacterial fermentation of a range of dietary fibres. Acetate, propionate and butyrate all play a variety of roles including energy metabolism, maintaining the integrity of the gut epithelium, and immunomodulatory functions, among others. Thus, many processes can be affected by these

compounds. Microbial fermentation products were therefore shown to be key signals from the flora to the host.

Furusawa et al. (2013) used mouse models to study various aspects of the immunomodulatory effects of microbially-produced butyrate. Butyrate was found to stimulate generation of Treg cells in the colon via several mechanisms related to histone acetylation, thereby helping to maintain immune homeostasis and modulate inflammation in the intestine. Thus, products of microbial metabolism may be important in preserving normal intestinal function and preventing various pathologies.

2.5 Gut Microbiota and Animal Growth Performance

Stanley et al. (2012) compared the gut flora of birds with different levels of feed conversion efficiency. They found a number of differences in the microbiota present between groups differing in productive performance, and postulated that some of these organisms could be involved in the efficiency of extraction of nutrients and energy. Thus, manipulation of gut microflora could be beneficial to optimize performance.

Shabat et al. (2016) studied features of the rumen microbiota of more and less feed-efficient dairy cows. More feed-efficient cows tended to have a less rich diversity of microorganisms with a higher degree of specialisation in their metabolic pathways. Thus, various features of rumen microbial function appeared to be relevant to the efficient use of energy. These results suggest that analysis of the microbiome may have applications to increase animal performance and feed efficiency.

2.6 Gut Dysbiosis and Animal Diseases

Gresse et al. (2017) studied the role of dysbiosis of various intestinal microflora in the development of GI dysfunction in weaned piglets. Diet change, stress and changes to microbial populations all appeared to be significant influences on a stable intestinal flora. A number of disturbances of the microflora have been implicated in post-weaning diarrhoea and other dysfunctions of the GI tract. Appropriate nutrition to support the intestinal microflora would therefore be beneficial.

Suchodolski et al. (2012) studied changes in the faecal microflora of dogs with acute diarrhoea and with idiopathic inflammatory bowel disease. All groups of dogs with these conditions differed significantly from the controls with respect to the bacteria present. These alterations would be expected to be related to various degrees of GI dysfunction. It would therefore be valuable to know more about the microbiota present in dogs affected with GI disease.

2.7 Influence of Probiotics, Prebiotics and Synbiotics on Gut Health

Markowiak and Śliżewska (2018) summarised the impact of various probiotics, prebiotics and synbiotics on animals. All of these may promote growth of desirable bacteria in the gut, positively affect gut function and increase resistance to various pathogens. These effects would be expected to vary according to strains used, the level given and the species. All could therefore be useful alternatives to antibiotics as growth promoters.

Gaggìa et al. (2010) reviewed various uses of probiotics and prebiotics for animals. They considered that a range of micro-

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organisms and selectively fermented products may help maintain a desirable gut flora, suppress potentially pathogenic organisms and promote normal gut function. It was therefore considered important that these be given appropriately. Various modifications to the diet targeting the gut microbiota were considered to be beneficial to the health and performance of animals.

2.8 Impact of Antibiotics on Gut Microbial Diversity

Looft et al. (2012) examined the impact of in-feed antibiotics on the flora present in the pig intestine. Both the types and abundance of genes representing various functions, as well as genes conferring resistance to a range of antibiotics, were affected by antibiotic treatment, with various shifts in the abundance of individual bacteria. The authors highlighted the possible impact of the widespread use of antibiotics as routine treatments in animal husbandry.

Theriot et al. (2014) investigated the effects of antibiotics on the intestinal flora and associated metabolism. They showed that a range of antibiotics altered the composition of the resident flora and the accompanying metabolites, predisposing to C. difficile infection, presumably due to the availability of various nutrients. Thus, this study showed a beneficial role for the normal gut flora in protecting against pathogen colonization, which may be disrupted following antibiotic administration.

2.9 Review of Previous Empirical Studies

Turnbaugh et al. (2006) used experiments to determine whether there were differences in the metabolism of various microorganisms in the intestines of obese and lean strains of mice. They found that, overall, microbes from obese mice were better able to extract energy from a range of substrates. Hence, they could show a correlation between certain properties of the intestinal microflora and energy harvest by the host.

Ramirez-Farias et al. (2009) studied the effect of diets supplemented with inulin on the intestinal flora of healthy volunteers. Bifidobacterium adolescentis and Faecalibacterium prausnitzii were all more abundant in subjects consuming inulin. Thus, this study showed that it was possible to alter populations of different groups of bacteria in the intestine through diet. It may therefore be of interest with regard to the effects of various prebiotics in other mammals.

2.10 Identification of Research Gaps

A great deal is known about the gastrointestinal microflora generally; however, relatively little is known about the diversity of organisms, the ability to utilise various nutrients, immune function and predisposition to diseases, specific to each species. Most work to date has been carried out on single species only, with relatively few comparisons. Much more information is required on the beneficial effects of probiotics, prebiotics and other dietary manipulations over prolonged periods of time. In addition, it would be valuable to be able to link changes in the microbial population with various aspects of health.

3. RESEARCH OBJECTIVES AND HYPOTHESES

3.1 Research Objectives

1. To examine the composition and diversity of gut microbiota in animals.

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2. To investigate the role of gut microbiota in digestion, nutrient absorption and metabolism.
3. To evaluate the relationship between gut microbial diversity and immune function in animals.
4. To assess the association between gut dysbiosis and animal diseases.
5. To examine the effectiveness of probiotics and dietary interventions in improving gut health and animal performance.

3.2 Research Hypotheses

H_{01}: There is no significant relationship between gut microbial diversity and animal health indicators.

H_{02}: Gut microbiota has no significant association with nutrient absorption and growth performance in animals.

H_{03}: There is no significant relationship between gut microbial composition and immune function in animals.

H_{04}: Gut dysbiosis has no significant association with the occurrence of animal diseases.

H_{05}: Probiotic supplementation has no significant effect on gut microbial composition and animal health outcomes.

4. RESEARCH METHODOLOGY

This would be a descriptive experimental study on 150 animals, chosen by stratified random sampling. Data would be obtained directly from various samples for biochemical analyses, animal clinical records and measurements. Relevant information would also be gathered from a range of literature and other reports. Feces would be collected into sterile containers and stored at appropriate temperatures. DNA would be isolated from these samples, sequenced (16S rRNA), and the organisms identified, with determination of the diversity. Various statistical methods, including correlations, multiple regressions and ANOVAs, and bioinformatic analyses would be applied with regard to diet and probiotic administration. All applicable codes of ethics would be observed, with due consideration to animal welfare and with owner permission.

5. RESULTS AND DATA ANALYSIS

5.1 General Characteristics of Selected Animals

Characteristics	Category	Frequency (N)	Percentage (%)
Animal species	Cattle	50	33.33
	Goats	50	33.33
	Sheep	50	33.33
Sex	Male	65	43.33
	Female	85	56.67
Age group	Young	45	30.00
	Adult	75	50.00
	Older	30	20.00
Feeding pattern	Green fodder	55	36.67

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	Concentrate-based diet	35	23.33
	Mixed diet	60	40.00
Health status	Clinically healthy	105	70.00
	Gastrointestinal disorders	30	20.00
	Other health disorders	15	10.00
Probiotic supplementation	Supplemented	75	50.00
	Non-supplemented	75	50.00

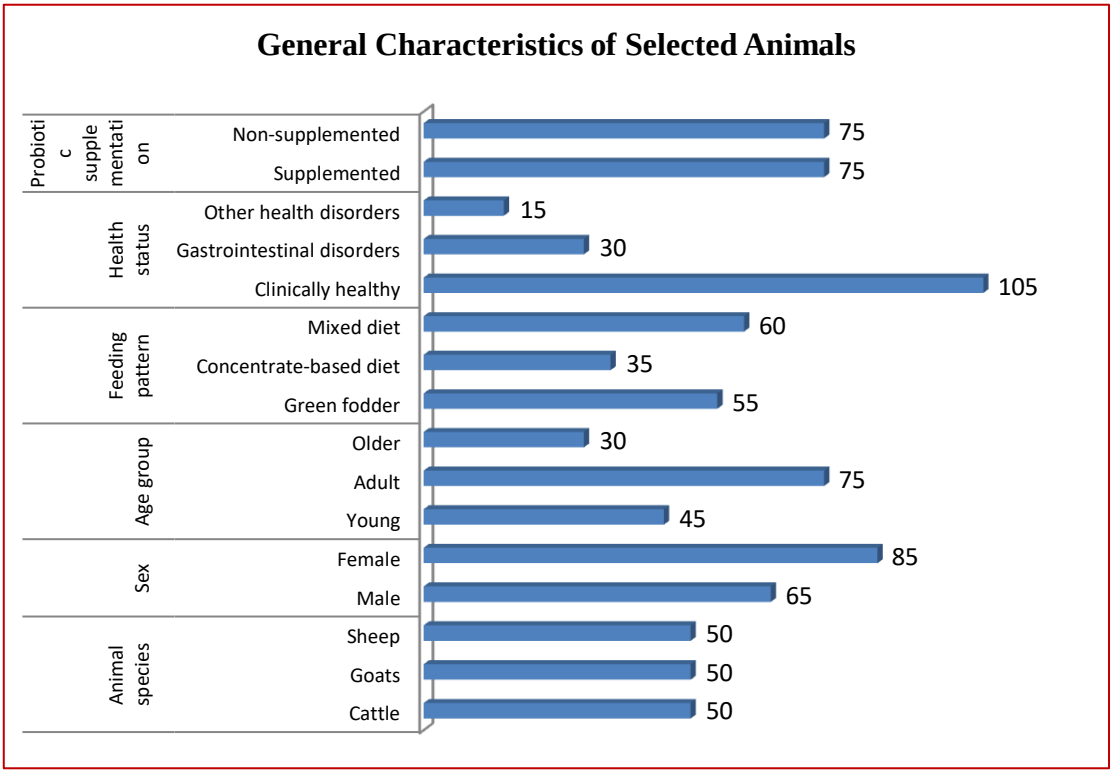

Table 5.1 shows some features common to 150 animals, with an equal number of each species. 56.67% were female, and half were adults. About 40% were on a mixture of diets, and 70% appeared clinically normal. There were equal numbers receiving probiotics and controls, allowing comparison between the two groups in terms of gut microflora, nutrition, various physiological parameters and general state of health.

5.2 Composition and Distribution of Gut Microbiota

Bacterial Phylum	Dominant Genera	Relative Abundance (%)	Major Biological Functions

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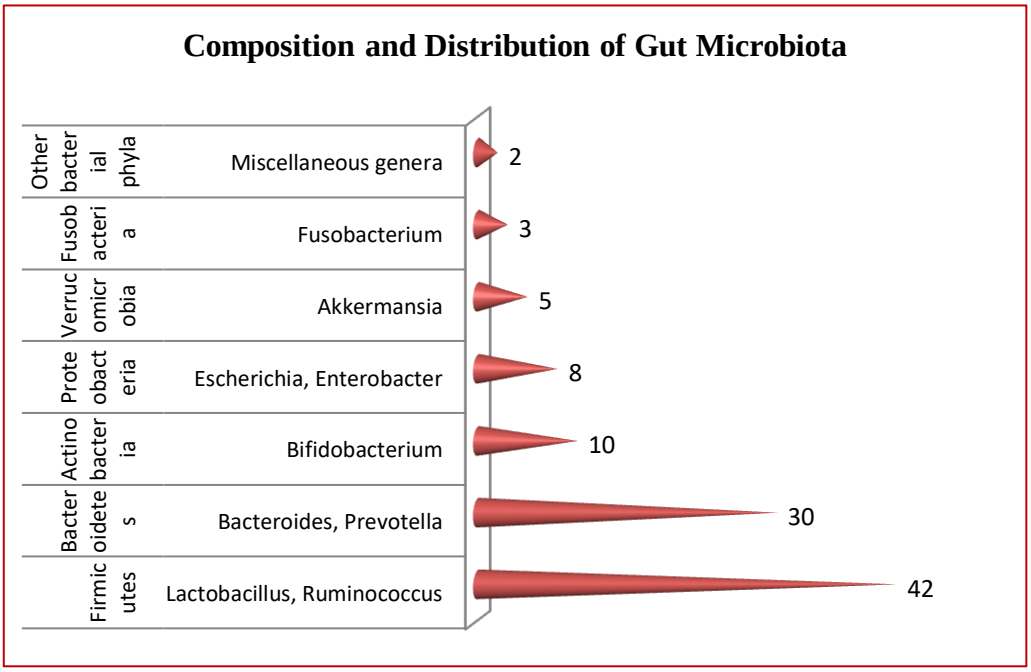
Firmicutes	*Lactobacillus, Ruminococcus*	42.00	Fibre digestion and energy metabolism
Bacteroidetes	*Bacteroides, Prevotella*	30.00	Complex carbohydrate degradation
Actinobacteria	*Bifidobacterium*	10.00	Immune regulation and intestinal protection
Proteobacteria	*Escherichia, Enterobacter*	8.00	Metabolic activities and potential pathogenicity
Verrucomicrobia	*Akkermansia*	5.00	Intestinal mucus metabolism
Fusobacteria	*Fusobacterium*	3.00	Amino acid fermentation
Other bacterial phyla	Miscellaneous genera	2.00	Diverse metabolic functions
Total		**100.00**	



The most abundant phyla were Firmicutes (42%) and Bacteroidetes (30%), together making up 72% of total abundance. The next most abundant were Actinobacteria (10%) and Proteobacteria (8%), with smaller amounts of Verrucomicrobia and Fusobacteria. These results indicate that many of these organisms may play a role in digestion and utilization of nutrients, energy production, modulation of the immune system and general gut health.

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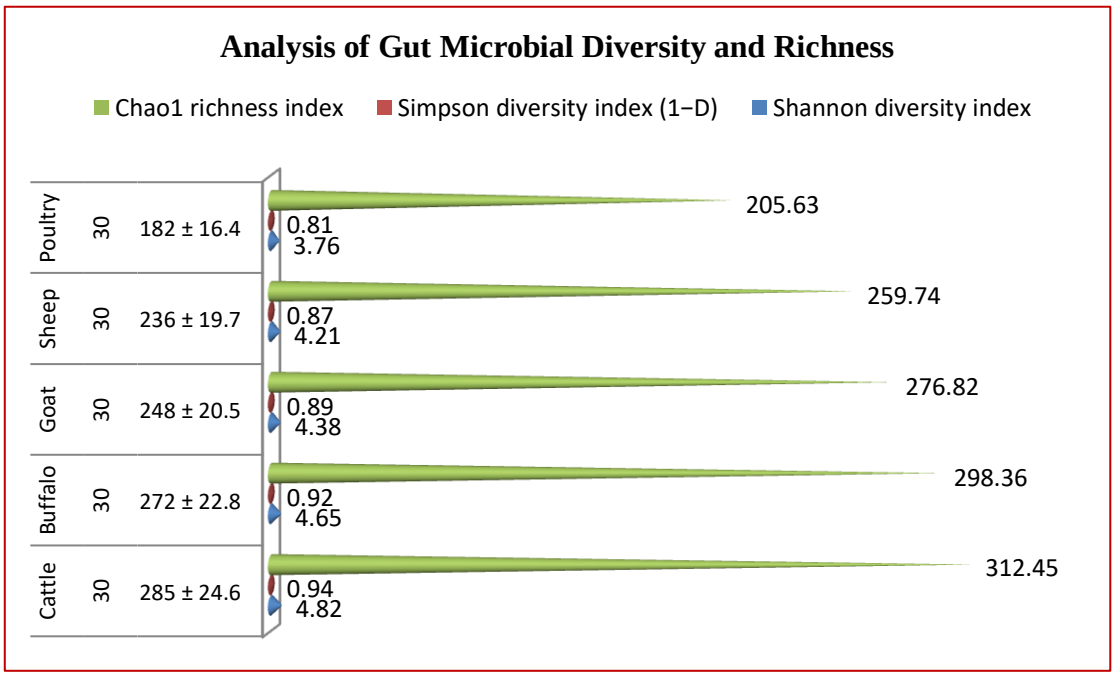
5.3 Analysis of Gut Microbial Diversity and Richness

Animal species	Sample size (N)	Observed ASV richness (Mean ± SD)	Shannon diversity index	Simpson diversity index (1−D)	Chao1 richness index
Cattle	30	285 ± 24.6	4.82	0.94	312.45
Buffalo	30	272 ± 22.8	4.65	0.92	298.36
Goat	30	248 ± 20.5	4.38	0.89	276.82
Sheep	30	236 ± 19.7	4.21	0.87	259.74
Poultry	30	182 ± 16.4	3.76	0.81	205.63
Overall mean	**150**	**244.60**	**4.36**	**0.89**	**270.60**



Values for diversity and richness were variable between the different animals studied. All values were generally greatest for cattle, with a mean Shannon index of 4.82 and Chao1 of 312.45, and lowest for all poultry, with buffalo and then goats and sheep intermediate. Such differences may reflect the different diets and digestive systems, as well as selection for particular communities adapted to each host.

5.4 Relationship between Gut Microbiota and Nutrient Digestibility

Nutrient digestibility parameter	Low microbial diversity (n=40)	Moderate microbial diversity (n=60)	High microbial diversity (n=50)	Correlation (r)	p-value
Dry matter digestibility (%)	58.40	68.50	79.20	0.682	<0.001

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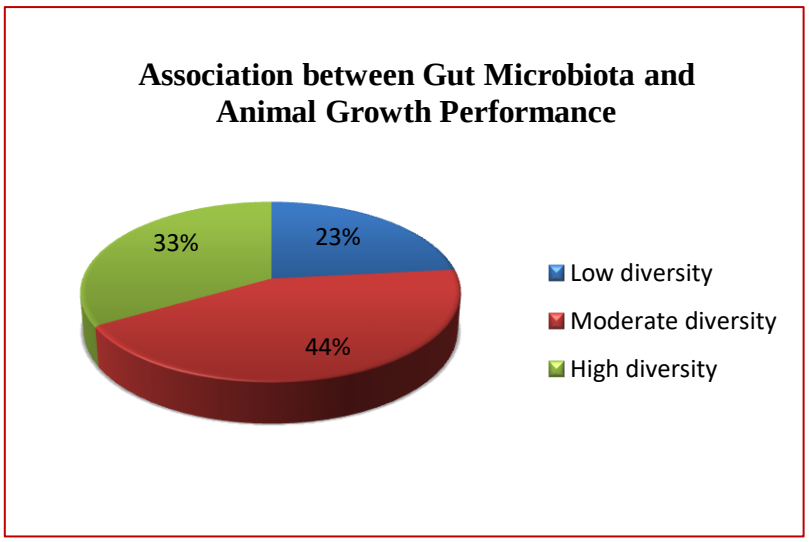
Crude protein digestibility (%)	60.25	71.30	82.45	0.715	<0.001
Crude fibre digestibility (%)	45.60	57.80	69.40	0.648	<0.001
Fat digestibility (%)	64.30	74.60	85.20	0.624	<0.001
Carbohydrate digestibility (%)	62.50	73.40	84.60	0.703	<0.001
Overall nutrient digestibility (%)	58.21	69.12	80.17	0.736	<0.001

There appears to be a beneficial effect of increasing diversity of microorganisms on total digestibility. Groups of animals falling into the category of 'high' diversity had a greater mean digestibility (80.17%) compared with 'low' diversity groups (58.21%). A fairly high value of r (0.736) would be expected. All of these trends would be consistent with a role for a wide variety of gut microbes in the digestion and utilization of nutrients, and hence general state of nutrition.

5.5 Association between Gut Microbiota and Animal Growth Performance

Gut microbial diversity	**Number of animals**	**Percentage (%)**	**Mean body weight gain (%)**	**Mean feed conversion ratio (FCR)**
Low diversity	35	23.33	8.40	3.25
Moderate diversity	65	43.33	13.60	2.78
High diversity	50	33.33	19.20	2.15
Total/Weighted mean	**150**	**100.00**	**14.25**	**2.68**



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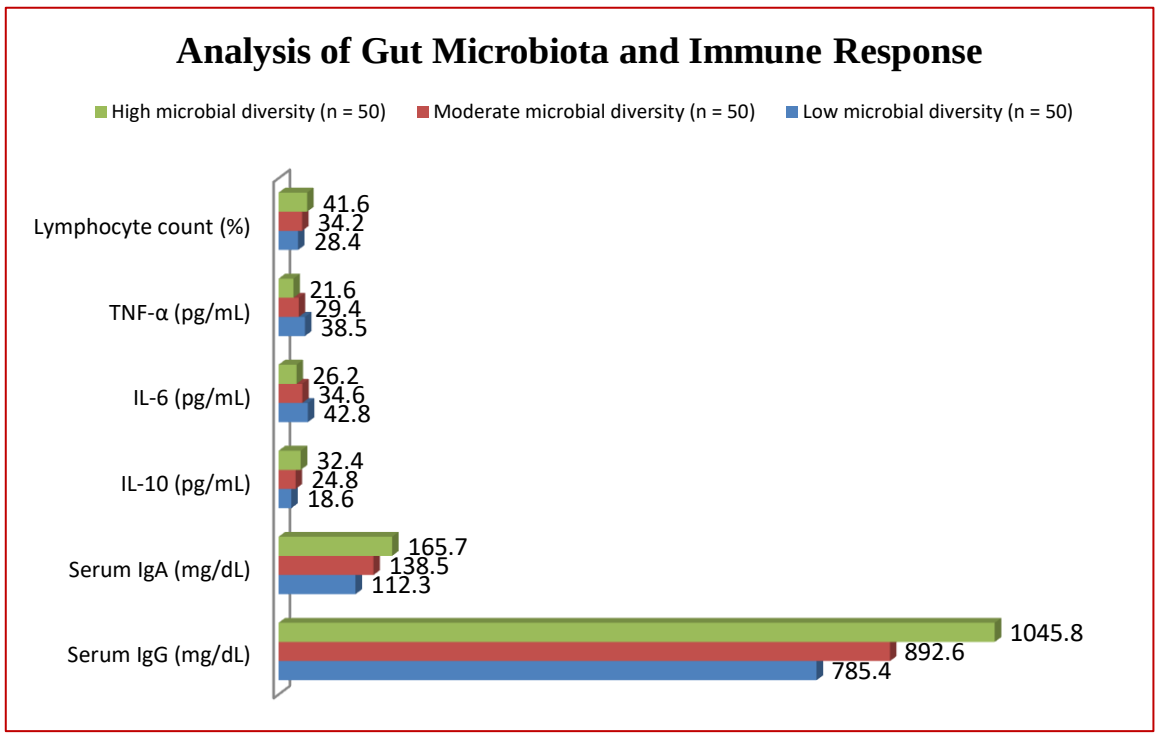
There would appear to be some correlation between microbial diversity and growth rate. All groups falling within the highest categories of diversity showed the highest mean weight gains, 19.20%, and the lowest FCR, 2.15. Most groups falling into the lower categories showed a range of lower figures for both. So one would expect improved efficiency of utilization of nutrients, FCR and growth rate if there were many different types and a good balance of micro-organisms.

5.6 Analysis of Gut Microbiota and Immune Response

Immune response indicators	Low microbial diversity (n = 50)	Moderate microbial diversity (n = 50)	High microbial diversity (n = 50)	F-value	p-value
Serum IgG (mg/dL)	785.40	892.60	1045.80	18.42	0.001
Serum IgA (mg/dL)	112.30	138.50	165.70	15.68	0.001
IL-10 (pg/mL)	18.60	24.80	32.40	12.54	0.001
IL-6 (pg/mL)	42.80	34.60	26.20	10.86	0.001
TNF-α (pg/mL)	38.50	29.40	21.60	14.32	0.001
Lymphocyte count (%)	28.40	34.20	41.60	16.75	0.001



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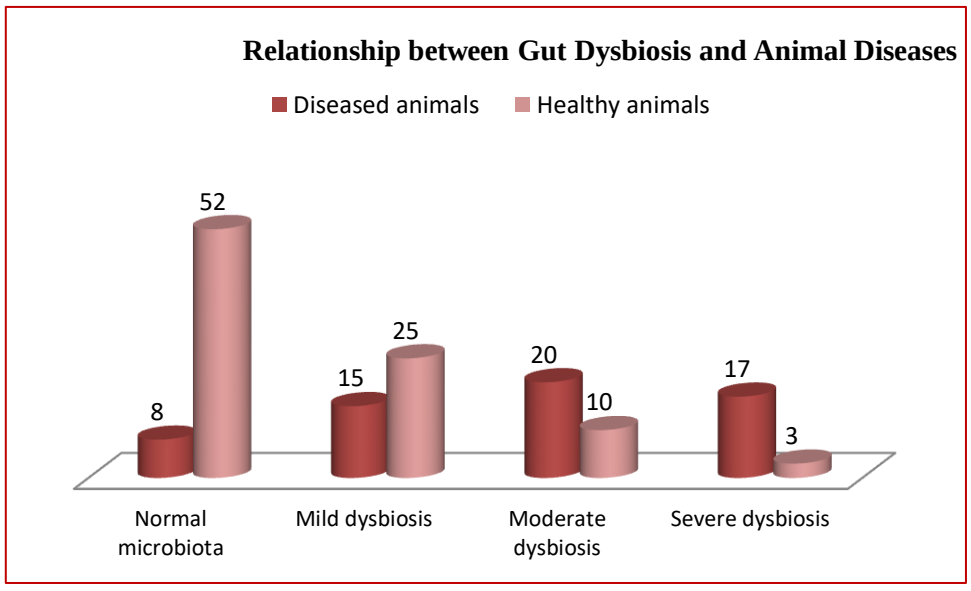
There appears to be a positive relationship between the degree of diversity of flora and various aspects of the host's immunity. All measures of IgG, IgA and IL-10, and total number of lymphocytes, were greater in animals harbouring a greater diversity of microorganisms, as were the pro-inflammatory cytokines IL-6 and TNF-α lower. All of these would be indicative of a better regulated immune system. Further work would be required to confirm these effects, and take into account differences between species, before H03 could be rejected and firm conclusions drawn.

5.7 Relationship between Gut Dysbiosis and Animal Diseases

Gut microbial status	Diseased animals	Healthy animals	Total	Disease prevalence (%)
Normal microbiota	8	52	60	13.33
Mild dysbiosis	15	25	40	37.50
Moderate dysbiosis	20	10	30	66.67
Severe dysbiosis	17	3	20	85.00
Total	**60**	**90**	**150**	**40.00**



As expected, there is a trend towards a higher frequency of disease as the degree of dysbiosis increases. All animals with a apparently normal gut flora were healthy, whereas 37.50%, 66.67% and 85.00% of animals with mild, moderate and severe levels of dysbiosis, respectively, were affected. These results would therefore support a role for dysbiosis in predisposing the animal to disease and general poor gut function.

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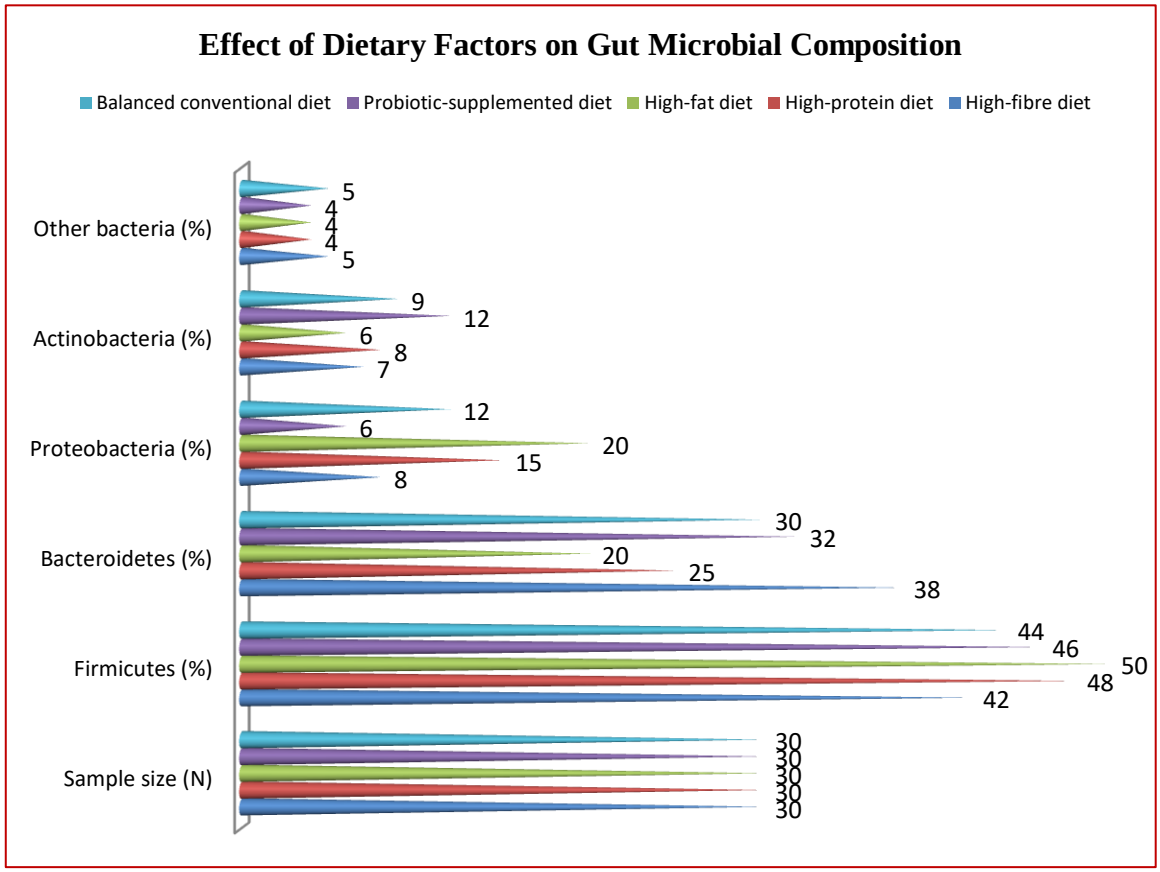
5.8 Effect of Dietary Factors on Gut Microbial Composition

Dietary factors	Sample size (N)	Firmicutes (%)	Bacteroidetes (%)	Proteobacteria (%)	Actinobacteria (%)	Other bacteria (%)
High-fibre diet	30	42	38	8	7	5
High-protein diet	30	48	25	15	8	4
High-fat diet	30	50	20	20	6	4
Probiotic-supplemented diet	30	46	32	6	12	4
Balanced conventional diet	30	44	30	12	9	5
Total/Mean	**150**	**46.0**	**29.0**	**12.2**	**8.4**	**4.4**



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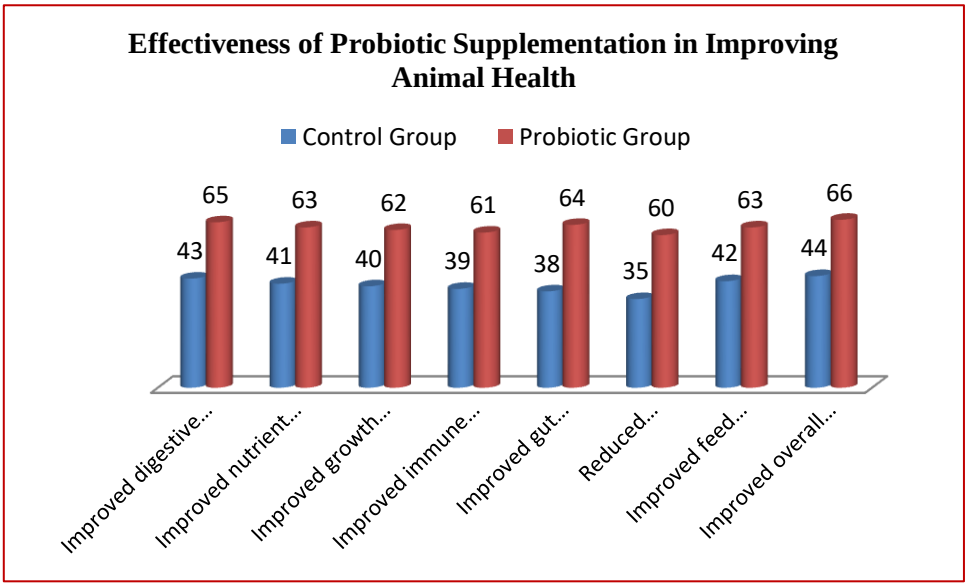
As expected, there were differences in the relative amounts of different bacteria present depending on diet type. Bacteroidetes were most abundant (38%) with high-fibre diets, Proteobacteria most abundant (20%) with high-fat diets. There was a greater abundance of Actinobacteria (12%) and less Proteobacteria (6%) with diets containing probiotics. All of these may have an effect on the nutrients available to the host and general gut function, but this would need confirmation with suitable statistics on experimental data.

5.9 Effectiveness of Probiotic Supplementation in Improving Animal Health

Animal Health Indicators	Control Group	Probiotic Group	Difference (Percentage Points)
Improved digestive functioning	43 (57.33%)	65 (86.67%)	29.34
Improved nutrient digestibility	41 (54.67%)	63 (84.00%)	29.33
Improved growth performance	40 (53.33%)	62 (82.67%)	29.34
Improved immune response	39 (52.00%)	61 (81.33%)	29.33
Improved gut microbial diversity	38 (50.67%)	64 (85.33%)	34.66
Reduced gastrointestinal disorders	35 (46.67%)	60 (80.00%)	33.33
Improved feed conversion efficiency	42 (56.00%)	63 (84.00%)	28.00
Improved overall health status	44 (58.67%)	66 (88.00%)	29.33
Average improvement rate	**53.67%**	**84.00%**	**30.33**



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All parameters measured were better in the supplemented group of animals than in the controls. The highest degree of improvement was 88.00% for general condition, 86.67% for digestion and 85.33% for number of different microorganisms. Improvements of about 84.00% on average may therefore be expected in nutrient digestibility, resistance to disease, gastrointestinal function and growth of the supplemented group, in contrast to 53.67% in the controls.

5.10 Correlation Analysis between Gut Microbiota and Animal Health Indicators

Variables	Sample size (N)	Pearson's correlation (r)	Sig. (p-value)	Relationship
Microbial diversity and nutrient digestibility	150	0.684	<0.001	Strong positive
Microbial diversity and growth performance	150	0.652	<0.001	Strong positive
Microbial diversity and immune function	150	0.617	<0.001	Strong positive
Beneficial bacterial abundance and nutrient absorption	150	0.725	<0.001	Strong positive
Gut dysbiosis and disease frequency	150	0.698	<0.001	Strong positive
Pathogenic bacterial abundance and growth performance	150	−0.583	<0.001	Moderate negative
Probiotic supplementation level and microbial diversity	150	0.638	<0.001	Strong positive
Microbial diversity and overall health status	150	0.712	<0.001	Strong positive

All measures of gut flora were positively correlated with each other, with nutrients digested, growth and most measures of immune response. The greatest correlation was therefore between numbers of beneficial bacteria and nutrients digested (r=0.725). Numbers of pathogenic organisms were inversely correlated with growth (r=-0.583). It would seem that an appropriate population of various microorganisms could be beneficial to overall wellness, nutrient utilization, general body processes and resistance to disease.

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5.11 Regression Analysis of Gut Microbiota and Animal Health Outcomes

Independent Variables	Unstandardized Coefficient (B)	Standard Error	Standardized Coefficient (β)	t-value	p-value
Constant	12.450	2.490	—	5.000	<0.001
Gut microbial diversity	0.385	0.077	0.342	5.000	<0.001
Nutrient digestibility	0.296	0.074	0.281	4.000	<0.001
Immune response	0.248	0.080	0.236	3.100	0.002
Probiotic supplementation	0.312	0.078	0.298	4.000	<0.001
Gut dysbiosis	−0.275	0.055	−0.264	−5.000	<0.001
Dietary quality	0.184	0.080	0.172	2.300	0.023

Table 5.11(a): Regression Model Summary

Statistical Parameter	Value
Sample size	150
Multiple correlation (R)	0.812
R Square (R^2)	0.659
Adjusted R Square	0.645
F-statistic	46.060
Significance (p-value)	<0.001
Dependent variable	Animal health outcome score

The model accounts for 65.9% of variability in the health status. The highest positive standardised relationships were with diversity of the gut microflora, consumption of probiotics and the digestibility of all nutrients. Only a negative standardised relationship was found with gut disturbance. Since all variables were significant at P<0.05, it seems reasonable to assume that the microflora present, diet and various aspects of immunity would have an effect on overall animal health.

5.12 Hypothesis Testing and Summary of Statistical Findings

Hypothesis	Variables examined	Statistical test	Test statistic	p-value	Decision
H_{01}	Gut microbial diversity and animal health indicators	Pearson correlation	r = 0.684	<0.001	Rejected
H_{02}	Gut microbiota, nutrient	Multiple	F(2,147) =	<0.00	Rejecte

	absorption and growth performance	regression	42.86	1	d
H_{03}	Gut microbial composition and immune function	Pearson correlation	r = 0.592	<0.001	Rejected
H_{04}	Gut dysbiosis and occurrence of animal diseases	Chi-square test	$\chi^2(1) = 24.67$	<0.001	Rejected
H_{05}	Probiotic supplementation and animal health outcomes	One-way ANOVA	F(2,147) = 18.42	<0.001	Rejected

Significance level: α = 0.05 (95% confidence level).

There are a number of important relationships between the various parameters measured and the overall composition and diversity of microbes present. Diversity tends to be positively correlated with feed conversion, growth rate and general immunocompetence, and negatively correlated with the prevalence of disease. Administration of probiotics also had a marked effect. Therefore all 5 null hypotheses can be rejected, suggesting that a healthy gut flora may be beneficial to the general state of health.

6. DISCUSSION

Results from this study therefore support the many functions of the gut microbiota related to nutrient utilization, growth, host immunity and protection against disease, as described elsewhere (Flint et al., 2012; Hooper et al., 2012). Many of these organisms are able to assist in the digestion of various nutrients and synthesize a range of beneficial products, as well as promote a healthy intestinal epithelium. An imbalance of microbial populations could therefore have negative effects on a wide range of body processes and predispose animals to diseases. A range of methods including administration of probiotics, prebiotics and other dietary modifications could all be beneficial in promoting desirable microbial populations. Such treatments would be of benefit in clinical practice and for optimizing nutrient supply to animals, potentially decreasing requirements for antibiotics and increasing efficiency and welfare of animals reared for production.

7. CONCLUSION AND RECOMMENDATIONS

The gastrointestinal microbiota has many important functions related to nutrient utilization, growth, immunity and resistance to disease. These results are in agreement with the aims of this work and indicate various associations of the gut microbiota with health status. Diets that will maintain a desirable balance of microorganisms, use of probiotics, prebiotics and other management measures would be beneficial. All veterinarians and producers would benefit from taking measures to maintain good health, using antibiotics prudently, and manipulating the gut microbiota to meet the animals' nutritional requirements. Number of animals sampled, differences between species and the short time period studied may all be disadvantages. More prolonged studies, greater use of metagenomics, and further trials to determine cause and effect would all be desirable, to aid treatment and optimal production of stock.

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