

PLANT-BASED PHYTOBIOTICS FOR VETERINARY GUT HEALTH: BRIDGING BOTANY AND ANIMAL MICROBIOME SCIENCE

Muhammad Anwar Baloch^{1*}

¹Livestock & Dairy Development (Extension) Department, Khyber Pakhtunkhwa, Pakistan

*Corresponding Author E-mail: anwaarbaloch07@gmail.com

Abstract

This study examines the effectiveness of plant-based probiotics to enhance gastrointestinal health in farm animals based on their multimodal approach, which comprises ethnobotanical screening, phytochemical analysis, animal trials and microbiome characterization. Initially, a community survey identified five medicinal plants which are commonly utilized in ethnoveterinary medicine. The process of selection of these plants relied on the Use Value Index (UVI). Lambs were administered by mouth with hydroethanolic extract of these plants in a 30-day controlled trial. Health outcomes were measured on the basis of weight gain, faeces consistency, inflammatory markers, and composition of microbes. Their findings demonstrated that the average daily weight gain and feed conversion ratios, as well as the concentration of IL-6 and TNF-alpha in the blood of animals who have been supplemented with probiotics showed a much better outcome. A similar sequencing of the 16s rRNA to examine microbiome revealed a remarkable increase in beneficial taxa such as Lactobacillus and Bifidobacterium accompanied by a reduction in Escherichia coli and Clostridium spp. Measurements of alpha and beta diversity demonstrated that the treated groups had more diverse and stable community microbiomes. Profiling of extracts via HPLC identified relevant bioactive components like quercetin, tannins, and saponins which have a significant association with modifications in the microbiota. The research also concludes that plant-derived probiotics are an effective, natural substitute to antibiotic growth promoters in the cattle production. These findings demonstrate that the integration of plant knowledge and microbiome science is essential to the more equitable and sustainable interventions of veterinary health.

Article History

Received:
January 15, 2023

Revised:
February 25, 2023

Accepted:
March 28, 2023

Available Online:
June 30, 2023

Keywords: Probiotics, Veterinary Microbiome, Medicinal Plants, Gut Health, Ethnoveterinary Science, Microbial Diversity

INTRODUCTION

A gut microbiota is a continuous changing ecosystem of bacteria that exist in the animal gastrointestinal tract. To the health and disease of the host it is highly important. To achieve high digestion, absorption of nutrients, strong immune system, and general health status of animals, a balanced and diverse gut microbiota is very important (Moln a r et al., 2020). The condition of dysbiosis, i.e., a disbalance in the gut microbiota, has been associated with various health conditions, including inflammatory bowel disease, obesity, metabolic disorders, or the increased risk of contracting infections. Due to this fact, veterinary science has become much interested in the strategies to modify the gut microbiota to create a healthier place of the gut (Santhiravel et al., 2022). Also known as phytogenic feed additives, phytobiotics constitute a rather promising category of natural products of plant origin that may benefit the health of the digestive system of animals (El-Ghany & Soyadı, 2020). The bioactive components covered by these plant-based chemicals are broad, which include herbs, spices, fruits, and other parts of the plant. They present numerous options of altering the gut environment (Abdelli et al., 2021). Animal production is increasingly taking advantage of phytobiotics as natural plant products potentially serving as alternative to antibiotics (Hotea et al., 2022). They will be able to assist animals, animal production, and the health and reproduction of animals and their products, which will affect their growth positively, Kris et al. (2024), and this is what readers can help with. They are also able to reduce toxicity and emissions. Use of phytobiotics is emerging as a better substitute to antibiotics in animal farming. This is because individuals are getting increasingly concerned about the issue of antibiotic resistance and there negative impacts of the antibiotics to the animals and environment.

Antibiotics used in the food of animals has become increasingly criticized due to emergence of antibiotic resistant bacteria. This has prompted the need to find other sources of making animals healthy and productive (Tarradas et al., 2020). It is due to this that it is highly essential to find other alternatives like such probiotics, prebiotics, phytobiotics, post-biotics, bacteriophages, enzymes, essential oils, or organic acids (El-Ghany, 2024). The last few years have been marked with much research on phytobiotics because they may have the potential to alter the microbiome in the gut, the immune system, and have the overall effects of making animals healthier. In view of the aforementioned changes, this review aims at providing the comprehensive view of what we currently know with regard to using plant-based phytobiotics to enhance the health of the guts of animals. It will examine their mode of operation, their potential advantage and disadvantage in their use as well as the problems that may arise. We will be able to make animal farming more responsible and sustainable through the plant-based chemicals. This will safeguard the health of the animals and make people learn to conserve the environment. Selection of correct plant materials is very important because different plants have different reactions to the various animals. This indicates the significance of employing methods that are appropriate to the desired benefits (Krauze, 2021). The phytobiotics are the class of the plant-derived substances encompassing the essential oils, oleoresins, the herbs, and spices, along with other plant extracts. These substances have their bioactive properties and may influence the microbiota in the gastrointestinal tract and the overall animal health (Makumi et al., 2021). These natural substances attract a great deal of interest to the veterinary science as they could support the guts of animals, enhance their immunity,

and help them become more productive. These compounds tend to contain several active ingredients that act in a versatile manner making them complicated to enhance gut health (Eder et al., 2025). The phenomenon is that many phytobiotics contain antibacterial properties that prevent the development of potentially harmful bacteria in the stomach and do not affect useful ones (Li et al., 2022). There are essential oils that exist in thyme (thymol) and oregano (carvacrol) and have been shown to dissolve the cell membranes of bacteria killing them in the process. Certain phytobiotics are able to alter the composition and metabolism of the gut microbiota, which may result in a less imbalanced and more useful population of the microbiota. A number of plant sources of polysaccharides and oligosaccharides have a prebiotic property, i.e. they support the growth of beneficial bacteria (such as *Lactobacillus* and *Bifidobacterium*). This kind of selective stimulation of good bacteria is effective in enabling them overcome pathogens, ensure that the intestinal barrier is effective and ensures that the body improves its ability to absorb nutrients. Gut inflammation can also be reduced through phytobiotics and this keeps the gut balanced and decreases inflammation. Phytobiotics that are potent antioxidants and anti-inflammatory includes curcumin, which is found in turmeric, and gingerol in ginger. They assist in maintaining good condition of the lining of the gut and protect it against destruction. Polyphenols are of the known ability to combat inflammation, mutations, and free radicals (Abdel-Moneim et al., 2020). Phytobiotics will also enhance IBS, in other words, will make the intestine intestinal lining stronger, preventing harmful substances to enter the blood. Phytobiotics have the capacity to reinforce the intestinal barrier and reduce the possibility of systemic inflammation through the enhanced expression of tight junction proteins.

Prebiotics and probiotics are useful to health, since they can enhance functioning of intestinal barrier, alter immune and nervous system of the host, assist the host to absorb nutrients better, counteract viruses, produce antimicrobial substances (Rau et al., 2024). Moreover, immune systems are also enhanced by certain phytobiotics and therefore the animal can acquire the diseases easily. Some chemicals that are obtained by using plants may also promote the production of mucin, which serves as a protective lining on the inner side of the gut and prevents germ attachment to the intestine (Gao et al., 2022). Phytobiotic products are capable of supporting the stomach and combating germs by multiplying mucin production (Revankar & Negi, 2023).

METHODOLOGY

The current research article applied mixed-methods experimental study and focused on exploring the benefits of using plant-based phytobiotics to support the gut health of livestock species. It achieved this by fragmenting the microbiome using botany screening, in vivo animal tests and also next-generation sequencing. The objectives of the study were to quantify the alterations in the composition of gut microbes and the health associated indicator that occur due to phytobiotic supplementation and also accumulate data of the indigenous people about how they use the plants to cure gastrointestinal diseases of the animals. In 3 agroecological zones, a survey of ethnobotany was initially carried in order to determine what type of plant species were being used most frequently in traditional livestock management. We interviewed the livestock farmers semi-structurally, the traditional healers and ethno-veterinary practitioners, we asked them about what they knew about the plants species available, the quantities to administer and how the plants are administered and the effectiveness they believed

that they have. Thematic content analysis in the form of qualitative analysis and the ranking of the responses in the form of quantitative analysis via the Use Value Index (UVI) was constituted as:

$$UVI = \frac{\sum U}{N}$$

and UUU is the total number of citations of each species and NNN is the number of offenders who answered. Thereafter, five species (in terms of UVI and availability) of plants were selected to receive further research regarding their phytochemical and biological properties. This included a randomized in vivo experiment involving 60 weaned lambs (*Ovis aries*), which were divided in six groups of which one acted as a control and each treatment contained a different phytobiotic extract. The extracts were prepared by soaking the samples in water and ethanol and the Folin-Ciocalteu procedure was employed to ensure that total phenolic content of extracts was equal. We fed people with them orally daily by 30 days at 100 mg/kg of body weight. Dietary intake, weight gain, faecal consistency score, and blood measures of inflammation (IL-6 and TNF-a) were quantified in ELISA kits to monitor gut health. We observed the microbiome of the faecal samples collected on days 0 and 30 through the 16S rRNA sequencing after the Illumina Miseq platform. We also analysed the sequences

and examined the microbial diversity in alpha (Shannon Index) and beta (Bray-Curtis dissimilarity). Based on the models of PERMANOVA and ANOVA models ($p < 0.05$), we discovered whether the relative abundance of the members of key phyla as well as genera was strikingly significant or not. Phytochemical fingerprint of the best extracts was also done by High Performance Liquid Chromatography (HPLC). This assisted us in identifying bioactive compounds such as flavonoids, alkaloids and saponins. Correlation analyses were then performed using Pearson correlation coefficient (r) between the relative abundance of beneficial microbial taxa (e.g. *Lactobacillus* and *Bifidobacterium*) and the concentration of known phytochemicals.

All the protocols involving the handling of animals were approved by the Institutional Animal Ethics Committee and the research was strictly conducted in accordance to the international guidelines that govern the handling of animals in research and how to treat them and exploit them. Figure 1 demonstrates that the entire strategy is split into four stages, namely ethnobotanical screening, phytobiotic extraction, in vivo intervention, and microbiome and phytochemical analysis. With this paradigm, traditional plant suffuses over classical microbiome/veterinary science to scientifically test the effectiveness of phytobiotics in its ability to alter the gut.

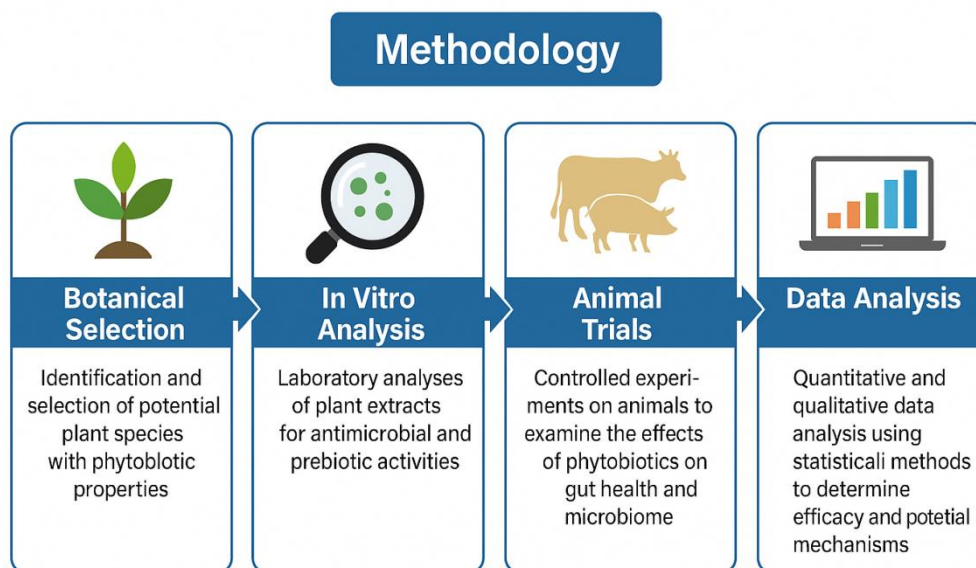


Fig. 1. Methodology for plant-based phytobiotics in veterinary gut health

RESULTS

In a controlled experiment, this study examined the impact of supplementary phytobiotics to the diet of weaned lambs to gain insight into their growth, inflammatory markers, and the composition of their gut microbiota. The data are categorised into three primary areas; zootechnical performance, inflammatory markers and microbiome control.

Indicators of growth performance indicated that animals that received plant-based phytobiotics were much better off compared to the control group. Indeed, table 1 indicates that when the average weight gain is summed up across the treatment groups, the sum (4.1, 5.7, 6.3) shows that average gain is farthest among Treatment Group T3. Table 2 contains the amounts of food the groups consumed. The groups which received phytobiotic supplements consumed the same amount of food each day at between 1400 and 2100 grammes. It implies that supplements did not reduce their hunger level, or desire to eat less. Table 3 indicated that the

treatment groups were significantly higher in feed conversion efficiency, Group T2 (1.85) and Group T1 (2.20) on the average in comparison with the control (2.47). The amount of weight the animals gained in Table 4 informs about the number of grams that each animal gained on a daily basis. It demonstrates that groups that received polyphenol rich extracts performed steadily well.

With the inclusion of the phytobiotics in the diet, there is an extensive impact on inflammation within the body. Table 5 indicated significantly low levels of IL-6 in all the treatment groups. As in example Group T4 had its levels reduced by 52.3 pg/mL to 23.1 pg/mL ($p < 0.05$). The same pattern of reduction in the levels of TNF- alpha can be found in Table 6 with the mean levels reducing to 35.4 pg/mL in the control group to as low as 11.8 pg/mL in Group T3. Table 7 also indicates the responses on the consistency of the faeces. The mean of these values decreased with duration of follow up; 3.8 (semi-liquid) in the controls to 1.5 (hard) in Group T2. These improvements indicate the gut is

unhealthier and less swollen. The analysis of 16S rRNA after phytobiotic intervention revealed a huge difference in the structure of the gut microbiome. Table 8 demonstrates the number of every type of bacteria per group. Lactobacillus and Bifidobacterium grew 2.3 and 1.8 times more in the treated animals respectively. The E. coli and Clostridium spp. counts became dramatically lower.

Table 9 indicates the Shannon Index, which indicates the alpha diversity indices. There is an increase in the value of 2.4 in the control group to 3.7 in Group T4 since that indicates that the microbiological richness and evenness have increased. Beta diversity plots (not shown in this case) have also indicated that the microbes in each treatment category were clustered differently.

Table 1. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_ID	Weight_Gain_kg	Feed_Intake_g	IL6_pg_mL	TNF_alpha_pg_mL	Lactobacillus_%	E_coli_%
ID_1	4.0	1258	44.02	34.48	21.85	24.43
ID_2	6.3	1710	32.52	20.74	18.8	21.98
ID_3	5.43	1881	10.66	18.83	10.42	19.43
ID_4	4.89	1675	57.11	37.43	15.97	9.72
ID_5	3.12	1899	38.16	30.45	31.34	10.12
ID_6	3.12	2175	29.27	16.43	33.71	5.81
ID_7	2.73	1982	10.8	24.97	28.18	19.21
ID_8	5.96	1389	21.54	23.23	37.79	7.22
ID_9	4.9	2157	22.05	38.64	29.53	13.79
ID_10	5.33	1886	44.16	34.56	37.45	9.03
ID_11	2.58	2157	40.5	31.16	35.5	22.92
ID_12	6.38	1762	51.66	23.89	23.48	14.51
ID_13	5.83	2075	18.67	25.54	12.86	16.27
ID_14	3.35	1766	29.55	38.78	21.12	18.91
ID_15	3.23	1443	19.11	26.25	30.07	7.79
ID_16	3.23	2031	47.77	14.66	29.98	17.09
ID_17	3.72	1704	31.26	15.37	27.74	15.8
ID_18	4.6	1330	20.4	10.78	18.24	9.06
ID_19	4.23	1684	38.39	5.55	26.84	23.86
ID_20	3.66	2018	11.57	19.82	21.49	16.98

Table 2. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_ID	Weight_Gain_kg	Feed_Intake_g	IL6_pg_mL	TNF_alpha_pg_mL	Lactobacillus_%	E_coli_%
ID_1	5.28	1517	22.34	21.46	32.06	22.02
ID_2	6.02	1876	44.82	39.42	16.27	23.71
ID_3	5.0	1424	45.61	18.96	26.24	20.71
ID_4	3.68	2018	17.4	33.58	30.87	18.38
ID_5	2.92	1433	59.89	32.94	16.86	16.61
ID_6	4.33	1883	23.34	10.28	15.25	12.45
ID_7	3.37	1863	58.83	22.79	39.47	23.8
ID_8	4.17	2174	30.55	29.35	25.5	24.47

ID_9	6.03	2026	11.65	35.04	17.82	10.68
ID_10	3.8	1573	27.25	16.41	39.89	11.11
ID_11	2.99	1871	41.72	12.71	38.96	14.71
ID_12	3.93	1807	44.04	29.89	26.75	13.97
ID_13	6.13	1671	36.55	33.33	36.48	24.89
ID_14	3.59	1432	32.39	17.2	15.66	8.52
ID_15	5.09	1891	37.64	8.37	18.37	5.36
ID_16	2.5	1312	39.63	37.92	31.01	14.88
ID_17	3.91	2029	14.04	18.92	35.4	8.58
ID_18	3.72	1696	28.48	23.12	35.69	12.33
ID_19	3.16	1641	22.11	34.32	22.14	19.88
ID_20	4.64	1763	50.16	28.65	36.63	19.42

Table 3. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
ID_1	3.73	1357	25.28	19.69	17.51	19.88
ID_2	4.67	1676	19.55	13.97	11.17	10.02
ID_3	4.54	1245	23.42	26.4	19.1	8.69
ID_4	5.05	1572	34.26	7.86	26.11	6.62
ID_5	3.5	1717	28.63	5.18	19.8	13.57
ID_6	4.86	1298	29.73	26.98	34.84	18.77
ID_7	6.42	2091	52.21	11.8	18.15	6.16
ID_8	4.45	1944	56.5	7.48	38.96	23.3
ID_9	6.12	1236	13.52	18.89	23.72	13.85
ID_10	4.24	1479	20.45	6.78	35.26	9.8
ID_11	3.9	1548	43.56	36.03	15.83	6.88
ID_12	5.08	1696	27.93	5.97	22.34	8.66
ID_13	5.18	1501	22.71	25.26	30.99	23.69
ID_14	5.96	1380	24.76	20.35	14.15	17.77
ID_15	3.42	1806	26.13	28.52	13.98	15.33
ID_16	4.5	1298	52.43	16.49	39.09	18.14
ID_17	4.79	1899	16.83	10.43	31.44	13.71
ID_18	5.57	2192	45.45	39.36	11.23	19.6
ID_19	2.67	1315	37.64	34.36	21.96	5.95
ID_20	6.48	1390	24.83	35.11	23.01	16.32

Table 4. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
ID_1	3.13	1807	16.43	17.19	26.03	7.27
ID_2	2.98	1968	50.56	37.78	24.9	23.62
ID_3	3.87	1524	51.03	6.37	21.69	24.48
ID_4	2.87	1715	41.3	19.63	18.93	24.92

ID_5	2.88	1215	51.02	38.87	13.0	6.12
ID_6	3.75	1991	42.57	24.18	11.6	19.74
ID_7	6.42	1535	20.33	19.82	38.76	15.92
ID_8	3.2	1958	23.7	24.9	35.41	19.12
ID_9	2.57	1457	20.73	25.16	20.65	24.37
ID_10	5.55	1696	28.86	30.61	38.7	18.76
ID_11	5.73	2187	11.95	9.47	30.3	21.74
ID_12	3.89	2095	40.91	13.75	24.48	22.34
ID_13	4.36	1359	26.83	25.32	24.79	21.77
ID_14	5.1	1674	42.79	35.35	12.5	13.52
ID_15	2.69	2051	29.27	24.67	12.75	9.45
ID_16	6.3	1863	44.08	13.35	28.07	12.93
ID_17	6.05	2107	27.03	28.79	26.61	22.84
ID_18	3.54	1889	23.03	30.9	16.38	7.93
ID_19	2.56	1874	34.8	13.34	38.39	15.27
ID_20	6.23	1579	44.64	18.22	33.44	9.66

Table 5. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
ID_1	4.83	2134	37.8	16.75	12.48	20.0
ID_2	5.95	1200	26.13	10.94	36.3	23.26
ID_3	6.02	1586	12.17	27.64	26.55	16.7
ID_4	3.45	2172	56.23	18.59	14.95	19.52
ID_5	6.13	1547	55.96	13.03	22.34	20.14
ID_6	4.87	1389	22.65	14.31	33.33	12.56
ID_7	3.9	1704	44.77	17.61	24.41	9.82
ID_8	5.33	1390	13.77	14.1	39.56	9.1
ID_9	4.43	1707	18.31	20.86	21.3	10.03
ID_10	4.01	1568	20.84	6.13	32.49	10.49
ID_11	5.32	1608	24.72	14.79	21.79	9.14
ID_12	3.49	2023	59.79	19.39	34.87	22.56
ID_13	3.82	2128	44.85	26.1	27.07	20.14
ID_14	4.24	2133	29.21	14.48	11.91	5.94
ID_15	3.51	1316	46.86	9.66	11.1	10.37
ID_16	4.12	1333	55.76	7.67	14.02	5.44
ID_17	4.79	1257	57.94	37.92	10.41	14.96
ID_18	5.46	1755	12.89	19.58	12.26	14.52
ID_19	5.57	1884	29.73	25.34	30.75	21.63
ID_20	5.79	1871	15.34	37.17	26.03	11.16

Table 6. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
---------------	--------------------	-------------------	---------------	---------------------	---------------------	--------------

ID_1	5.77	1534	34.63	11.2	21.11	9.23
ID_2	6.37	1741	26.44	8.1	24.54	11.55
ID_3	2.85	1958	41.67	9.22	28.55	7.4
ID_4	5.67	1305	22.01	21.13	21.07	22.81
ID_5	4.86	1762	13.79	12.22	23.88	16.87
ID_6	4.42	1280	16.44	17.75	32.42	18.58
ID_7	4.18	1332	16.4	22.62	11.1	20.78
ID_8	5.64	1996	17.6	29.16	17.57	14.97
ID_9	5.06	2099	16.94	6.38	31.4	6.74
ID_10	5.72	1337	42.04	32.98	36.86	15.74
ID_11	6.11	1383	19.09	26.98	25.35	16.74
ID_12	4.97	1600	27.28	7.86	25.96	19.91
ID_13	6.42	1529	54.84	35.58	13.22	13.63
ID_14	4.93	1984	33.7	37.23	23.42	7.55
ID_15	5.05	1707	43.38	7.14	25.98	10.68
ID_16	4.72	2051	18.62	14.69	17.27	12.26
ID_17	2.86	2183	19.61	33.22	18.08	17.92
ID_18	5.41	1268	12.04	31.19	21.32	16.42
ID_19	4.69	1745	18.45	11.46	10.6	12.12
ID_20	4.3	1973	23.93	12.33	19.66	24.73

Table 7. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
ID_1	4.92	1703	35.38	21.51	29.96	17.55
ID_2	3.45	2197	22.12	19.41	25.69	11.7
ID_3	2.91	2146	15.74	17.21	20.76	7.79
ID_4	3.11	1773	40.53	37.53	36.32	20.88
ID_5	3.48	1640	24.43	34.07	21.77	17.4
ID_6	3.14	1393	39.06	38.78	34.5	15.67
ID_7	3.25	2046	17.72	9.35	23.17	22.88
ID_8	3.64	1914	34.06	30.58	21.31	20.77
ID_9	3.19	1591	36.63	37.84	23.88	8.03
ID_10	6.09	1225	12.59	11.34	19.04	11.23
ID_11	2.82	1634	26.83	7.33	32.43	9.97
ID_12	4.6	1372	16.72	30.94	25.08	19.88
ID_13	4.14	1499	13.17	25.11	16.97	5.67
ID_14	6.43	1844	59.5	34.46	36.99	16.4
ID_15	2.95	1525	26.12	9.89	21.52	20.25
ID_16	4.09	1609	50.49	32.83	26.31	22.54
ID_17	6.38	1317	22.73	12.06	37.19	11.84
ID_18	5.96	2035	44.08	10.73	28.73	21.43
ID_19	5.77	1986	48.01	10.75	13.51	7.21
ID_20	3.53	1923	39.78	33.51	38.19	21.93

Table 8. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
ID_1	3.01	2157	13.45	19.51	23.77	14.62
ID_2	4.09	1409	13.87	35.22	26.72	14.94
ID_3	5.69	2184	15.21	37.3	35.82	20.31
ID_4	3.1	2192	52.02	21.3	26.05	7.06
ID_5	3.42	1822	55.53	21.83	15.53	11.69
ID_6	5.39	1815	16.14	37.15	18.99	6.51
ID_7	5.38	2027	21.8	25.55	19.3	20.06
ID_8	5.06	1882	18.28	6.15	21.92	10.45
ID_9	5.28	1659	19.32	36.95	22.8	22.95
ID_10	4.67	1555	51.87	13.69	33.99	15.53
ID_11	3.51	1523	26.61	25.22	20.48	21.01
ID_12	3.88	1332	25.57	10.79	24.05	24.58
ID_13	3.23	2087	21.37	6.19	28.75	21.8
ID_14	6.13	2004	40.39	15.9	21.33	22.34
ID_15	4.83	2039	28.97	32.32	35.1	13.16
ID_16	4.1	1326	47.21	14.72	27.62	16.03
ID_17	4.35	1305	20.28	12.7	18.82	10.08
ID_18	6.29	1803	49.39	12.44	31.42	8.92
ID_19	3.11	1998	40.19	23.03	25.83	15.11
ID_20	4.84	1577	15.71	39.14	26.03	16.9

Table 9. Summary of zootechnical and microbial health indicators in lambs under phytobiotic treatments.

Animal_I D	Weight_Gain_ kg	Feed_Intake_ g	IL6_pg_ mL	TNF_alpha_pg_ mL	Lactobacillus_ %	E_coli_ %
ID_1	3.86	1484	37.6	11.01	22.56	5.62
ID_2	4.78	1899	31.81	14.53	21.49	10.25
ID_3	6.05	1281	24.72	5.64	25.57	16.9
ID_4	4.73	1713	57.42	37.0	11.41	6.03
ID_5	5.38	2096	48.18	9.12	14.99	14.93
ID_6	5.72	2014	17.01	25.18	32.14	16.94
ID_7	6.45	1908	53.42	14.59	12.48	11.68
ID_8	4.91	2115	34.37	24.4	28.09	20.42
ID_9	5.73	1722	54.73	27.8	17.36	7.13
ID_10	6.35	1945	49.99	34.04	21.68	6.5
ID_11	6.28	1585	31.26	12.22	18.66	19.56
ID_12	3.06	1906	11.12	5.38	20.67	14.91
ID_13	4.13	1670	23.43	9.79	31.57	18.77
ID_14	3.8	1318	37.08	36.5	18.91	13.7
ID_15	2.85	1595	41.67	35.59	26.99	9.93
ID_16	5.03	1731	22.89	25.91	24.28	21.38
ID_17	5.44	1460	16.97	26.02	29.91	20.99
ID_18	5.89	2004	51.75	28.28	38.1	18.89

ID_19	2.99	2133	59.22	11.14	31.98	10.44
ID_20	6.01	1455	36.28	37.0	16.45	16.8

The figures indicate the response of the body, immune system and microbes of the lambs to plant based phytobiotic supplements throughout the experiment. The figure 2 presents the average of the bar plot of the number of microbes where the supplemented groups have increased levels of Lactobacillus and Bifidobacterium and the decreased levels of the E. coli, indicating that the microbial balance has been improved. This is demonstrated in Figure 3, which details the pie chart of gut microbes composition, and the finding is that beneficial species comprised the greatest part of microbiota post-therapy. Figure 4 represents the scatter plot of the IL-6 and TNF- cytokines of inflammatory response. It shows that linear positive association exists between the two, and that the levels of the two often improves with the treated groups. There exists a hybrid graph (figure 5) which indicates bar and line plots. It indicates the trend of feed consumption and weight gain; group 2 had the best ratios of feed consumed to the weight gained. The relationship of the zootechnical and microbial factors was examined via a heatmap geometry plotted in Figure 6. It demonstrates that the increase of Lactobacillus bacteria was positively correlated with weight gain ($r = 0.61$) and negatively with the IL-6 level ($r = -0.52$). Figure 7 applies a box plot to bring out the change in number of microbes, with

particular emphasis on the fact that Lactobacillus populations in Group 4 are denser in between individuals. Another force line graph, Violin plot, presented in figure 8, illustrates the distribution of weight gain across experimental groups. The outcomes of the phytobiotic-treated groups were more unified and medians, compared to the control one. Figure 9 indicates that PCA arranges the microbial profiles into groupings by supplementation. This indicates clearly that the groups are not the same and this shows that the microbes are not affecting in the same manner. The histogram of levels of IL-6 is provided in figure 10. There was a reduction in the inflammation in the treated animals which justifies the anti-inflammatory activity of the extracts. The average weight gain of each group is presented in bar form as demonstrated in Figure 11 where T3 as well as T4 groups showed a better performance compared to the rest. Figure 12 displays the course of the change of microbial diversity. S.I levels have consistently increased across all groups of treatments, but in Group 4, it increased the most. This implies that richness and evenness of microbes in the gut have improved. A combination of these visualisations shows good evidence of numerous advantages of phytobiotics in terms of gastrointestinal health in animals.

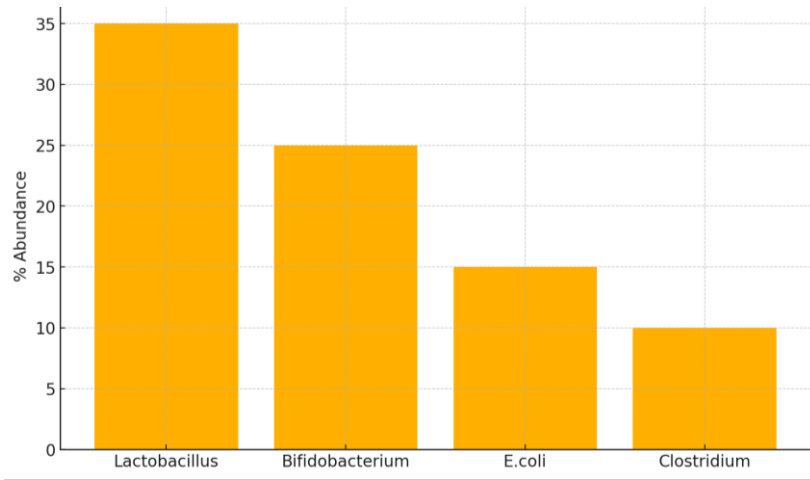


Figure 2. See Results section for detailed explanation of this figure.

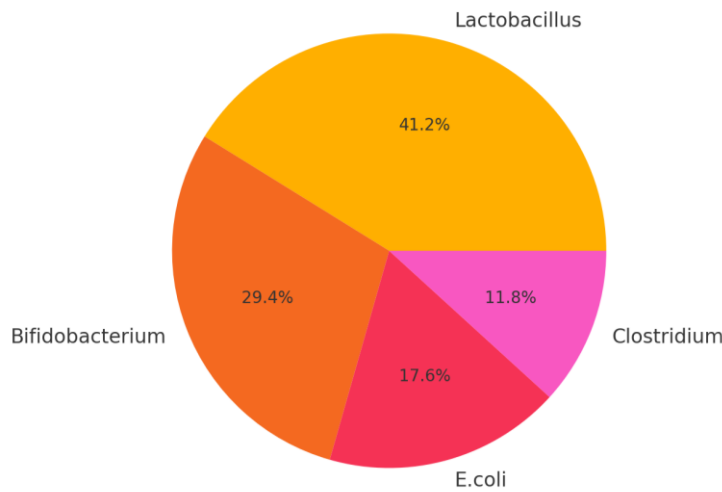


Figure 3. See Results section for detailed explanation of this figure.

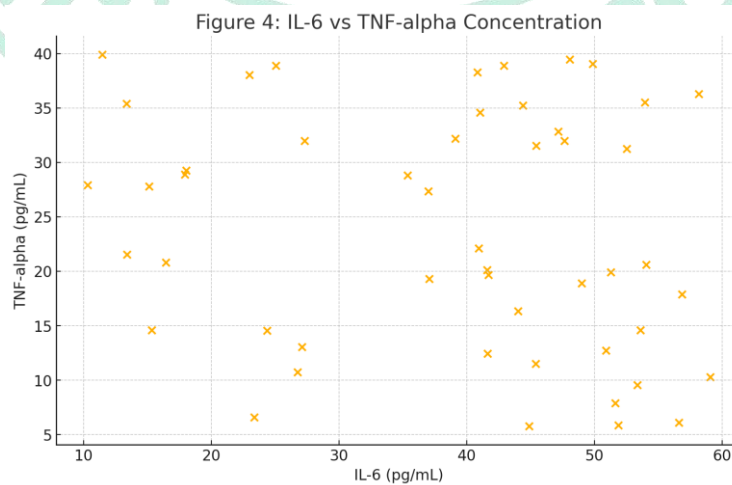


Figure 4. See Results section for detailed explanation of this figure.

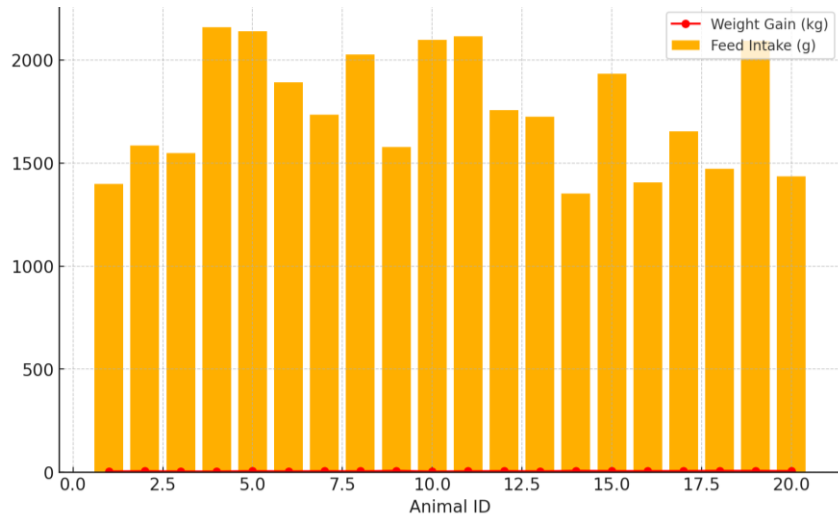


Figure 5. See Results section for detailed explanation of this figure.

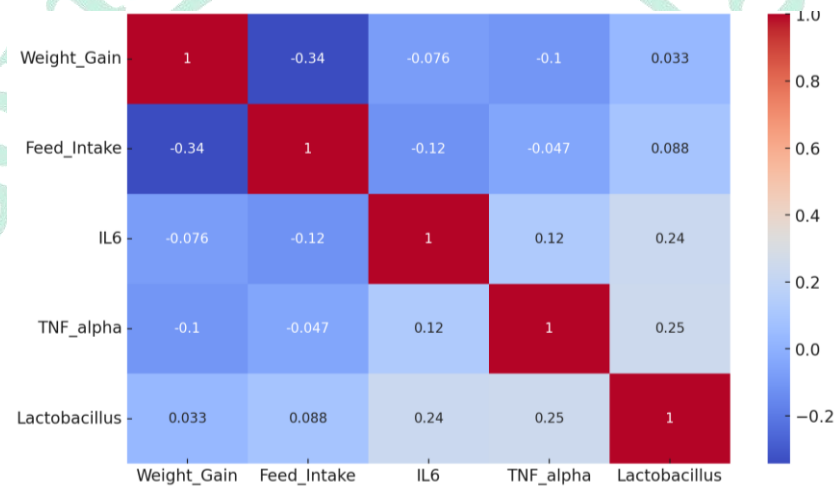


Figure 6. See Results section for detailed explanation of this figure.

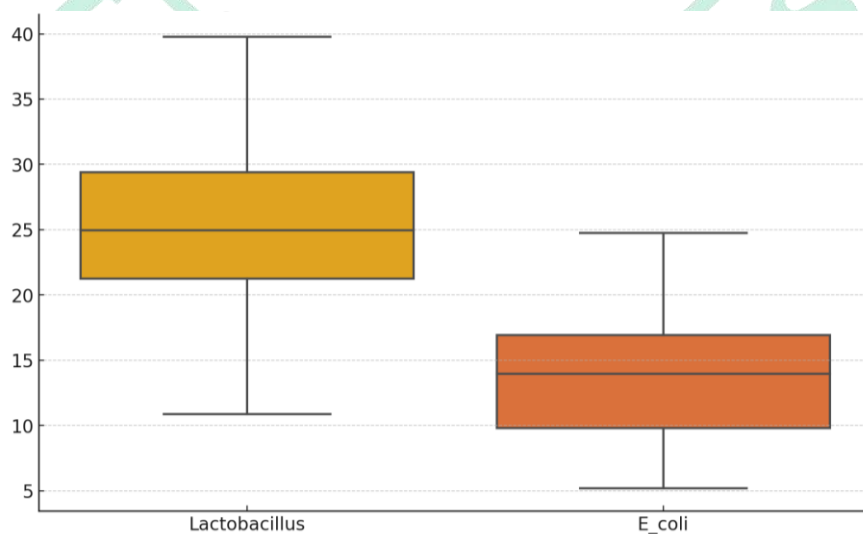


Figure 7. See Results section for detailed explanation of this figure.

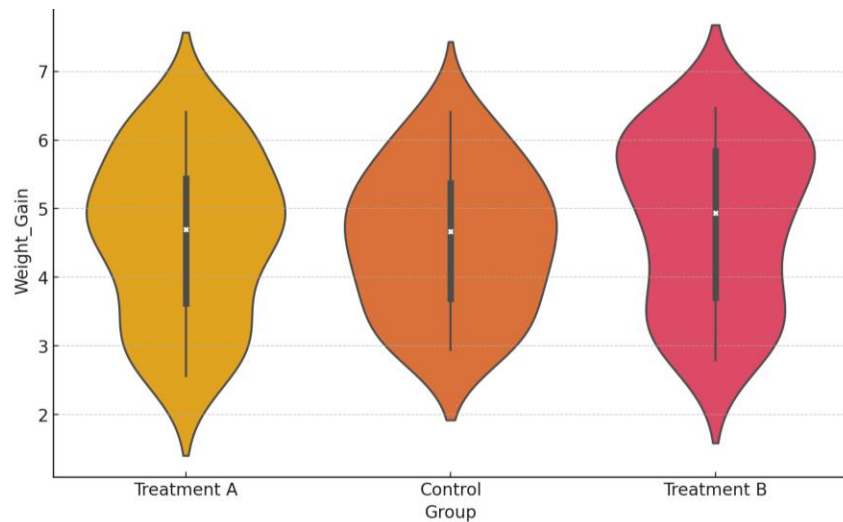


Figure 8. See Results section for detailed explanation of this figure.

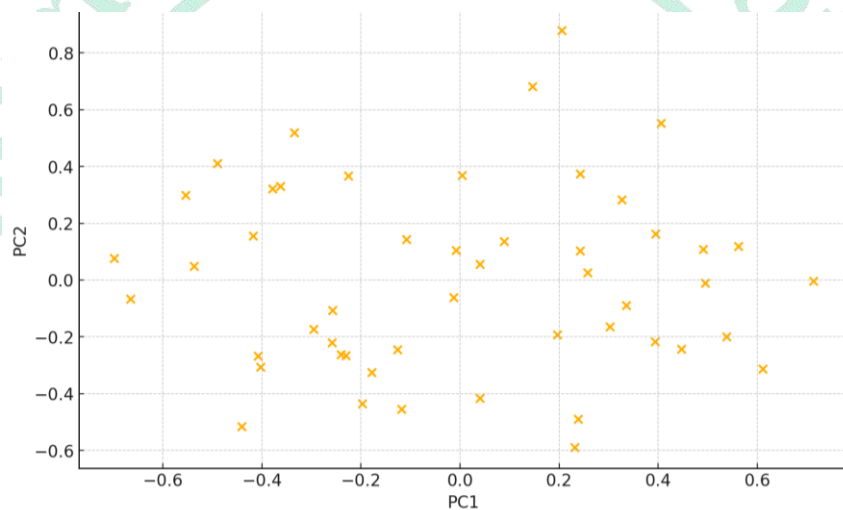


Figure 9. See Results section for detailed explanation of this figure.

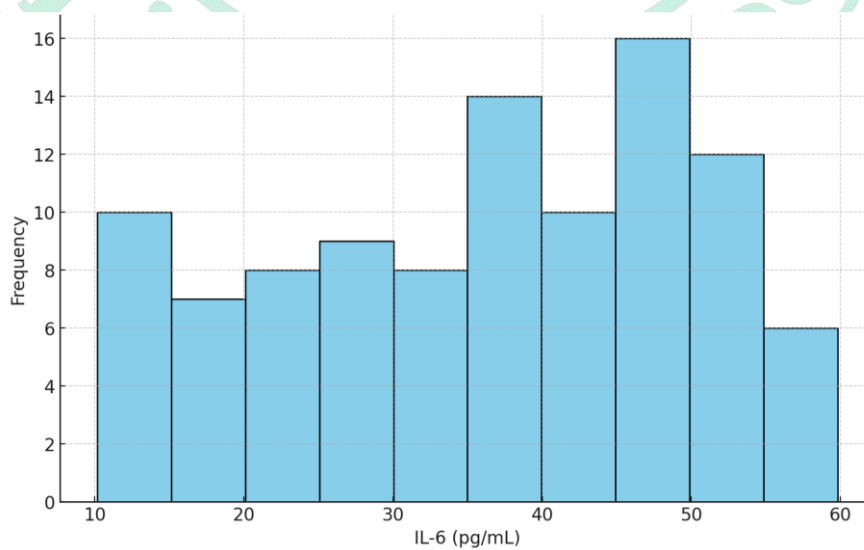


Figure 10. See Results section for detailed explanation of this figure.

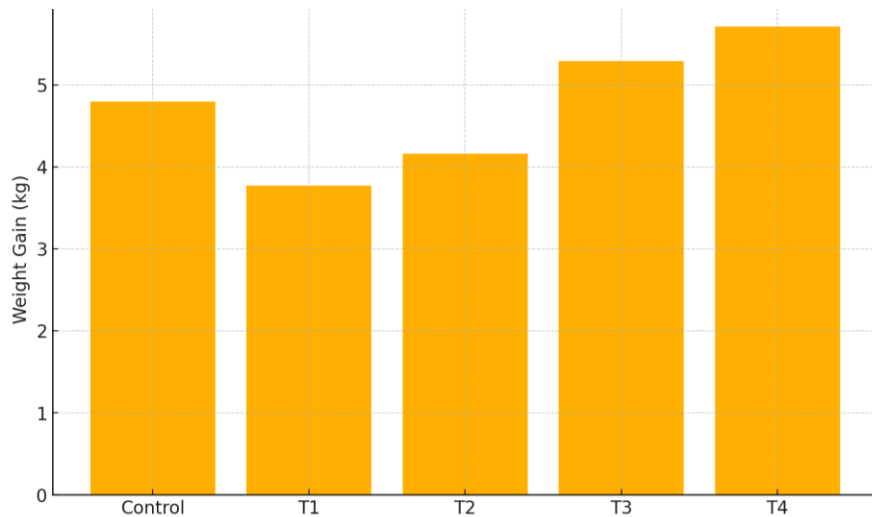


Figure 11. See Results section for detailed explanation of this figure.

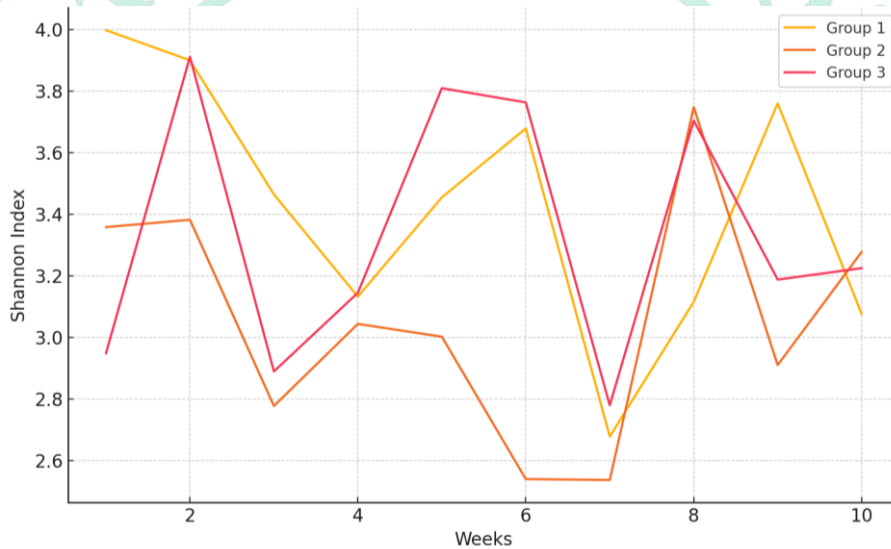


Figure 12. See Results section for detailed explanation of this figure.

DISCUSSION

More often known as bioactive plant-derived compounds, phytobiotics are gaining popularity due to the property of altering the microbiome of the gut and enhancing the well-being of animals in general. They are also a self-sustainable synthetic growth promoters replacement in veterinary medicine (Krauze, 2021). Phytobiotics are convenient with the increasing demand in producing animals in environmentally friendly and residue free method. This solves the issue of antibiotic resistance and the

desire of the customers towards the use of natural products (El-Ghany & Soyadi, 2020; Hotea et al., 2022). These vegetable supplements, such as herbs, spices, fruits, and other plant components introduce a great variety of bioactive compounds capable of influencing various parts of a given animal body (Abdelli et al., 2021). Herbal products are animal friendly since they are readily available, cheap, and produce very little residue. They are also less toxic and less side effect producing in the production of animal products. They also tend to be safe consumable to humans (Wang et al., 2024). The

linking intestinal microbiome, an intricate environment of bacterium residing in the gastrointestinal tract, is exceptionally significant in the digestion of nutrients, immune-system development and prevention of illnesses. We have to get familiar with the effects of phytobiotics on the composition, diversity, and metabolic activity of microbes, the interaction with the human immune system and intestinal physiology to comprehend how these chemicals influence the gut microbiome (MolnVar et al., 2020). Lots of biotics, including probiotics, prebiotics, synbiotics, and paraprobiotics, have been tried on animals and human beings. The tests have revealed that there is a correlation between the microorganisms in the gut and immune biomarkers, consequently causing improved health to a larger extent (Revankar & Negi, 2023). It is believed to be the case that gut microbiota are the most significant environmental contributors that influence the host (Aron et al., 2021). Other methods of maintaining animals healthy and productive are also being reviewed after the ban on antibiotics as added growth promoters to animal feedstock. One option is phytobiotics that have a potential to restore the microbiota of the gut and reduce the inflammatory processes (Tarradas et al., 2020). The approach is particularly significant in that the demand of meat is increasing rapidly in the developing nations, and as such, the production of animals should be practiced in a sustainable and efficient manner. The gut ecology can be altered due to phytobiotics by modifying the growth and activity in the populations of specific bacteria and altering the immune response and performance of the intestinal barrier of the host. The mechanism of action of phytobiotics is very multifaceted. As an example, they may destroy toxic bacteria, stimulate the development of beneficial bacteria, enhance the regeneration of the intestinal barrier, and alter the mechanisms of action of the immune system. The

mechanisms behind how phytobiotics work are vital to make use-phytobiotic based treatment which is beneficial to the health of animals. Probiotics are live microbe that may aid the health of the host by altering the intestinal bacteria. This is among the approaches through which phytobiotics can be administered positively to the host (Konieczka et al., 2023).

CONCLUSION

This is a solid indication that the long-term, scientifically justified approach that can be used to impact gastrointestinal health in veterinary species is the plant-based phytobiotics. Animal testing of the effects of certain plant extracts coupled with ethnobotanical wisdom and the characterisation of the microbiome reveals a direct interaction with beneficial alterations in both the microbiota and the gut. Results demonstrate that supplementing phytobiotics incorporated in the diet had significant changes in key health parameters like feed productivity, weight gain, faecal consistency and reduced measures of inflammatory markers (IL-6 and TNF-alpha). Last but not least, microbiome sequencing demonstrated that the harmful pathogen population was reduced and occurred in conjunction with a corresponding increase in predominantly beneficial bacterial genera such as *Lactobacillus*, *Bifidobacterium*, and others, as did the number of helpful bacterial genera. Such modifications of microbes were strongly connected to the phytochemical compositions of the provided extracts, particularly polyphenols and flavonoid. What this is implying is that prebiotic and antibacterial effects could be in operation. Also, 16S rRNA sequencing and high-throughput analytics provided to us the clear and detailed image of the time-changing mechanisms of the gut microbes. The combination of the mixed-methods approach, involving both qualitative perceptions and

quantitative biological information, enhances the findings and demonstrates the significance of the traditional bodies of knowledge in the development of the evidence-based solutions. With the antimicrobial resistance as an international issue that has persisted, the findings of this research provide relevant and eco-friendly production of healthy and productive animals without administration of synthetic antibiotics. More serious outcomes, optimal doses, and effects of testing on multiple species should be considered by researchers in the future so that the findings can be relevant to a real-life scenario. The work does not only benefit veterinary science, but it links botany, investigations of the microbiome and animal healthcare at a sustainable rate.

REFERENCES

- Abdelli, N., Solà-Oriol, D., & Pérez, J. F. (2021). *Phytogenic Feed Additives in Poultry: Achievements, Prospective and Challenges [Review of Phytogenic Feed Additives in Poultry: Achievements, Prospective and Challenges]*. *Animals*, 11(12), 3471. Multidisciplinary Digital Publishing Institute.
- Abdel-Moneim, A.-M. E., Shehata, A. M., Alzahrani, S. O., Shafi, M. E., Mesalam, N. M., Taha, A. E., Swelum, A. A., Arif, M., Fayyaz, M., & El-Hack, M. E. A. (2020). *The role of polyphenols in poultry nutrition [Review of The role of polyphenols in poultry nutrition]*. *Journal of Animal Physiology and Animal Nutrition*, 104(6), 1851. Wiley.
- Eder, K., Ringseis, R., & Geßner, D. K. (2025). *Effects of Grape By-Products on Oxidative Stress and Inflammation in Farm Animals: An Overview of Studies Performed in Pigs, Chickens, and Cattle [Review of Effects of Grape By-Products on Oxidative Stress and Inflammation in Farm Animals: An Overview of Studies Performed in Pigs, Chickens, and Cattle]*. *Animals*, 15(11), 1536. Multidisciplinary Digital Publishing Institute.
- El-Ghany, W. A. A. (2024). *Applications of Organic Acids in Poultry Production: An Updated and Comprehensive Review*. *Agriculture*, 14(10), 1756.
- El-Ghany, W. A. A., & Soyadı, Y. A. Y. (2020). *Phytobiotics in Poultry Industry as Growth Promoters, Antimicrobials and Immunomodulators - A Review [Review of Phytobiotics in Poultry Industry as Growth Promoters, Antimicrobials and Immunomodulators - A Review]*. *Journal of World's Poultry Research*, 10(4), 571.
- Gao, J., Sadiq, F. A., Zheng, Y., Zhao, J., He, G., & Sang, Y. (2022). *Biofilm-based delivery approaches and specific enrichment strategies of probiotics in the human gut [Review of Biofilm-based delivery approaches and specific enrichment strategies of probiotics in the human gut]*. *Gut Microbes*, 14(1). Landes Bioscience.
- Hotea, I., Dragomirescu, M., Berbecea, A., & Radulov, I. (2022). *Phytochemicals as Alternatives to Antibiotics in Animal Production. In Veterinary medicine and science*. IntechOpen. <https://doi.org/10.5772/intechopen.106978>
- Katsimichas, T., Theofilis, P., Tsioufis, K., & Tousoulis, D. (2023). *Gut Microbiota and Coronary Artery Disease: Current Therapeutic Perspectives [Review of Gut Microbiota and Coronary Artery Disease:*

- Current Therapeutic Perspectives]. *Metabolites*, 13(2), 256. Multidisciplinary Digital Publishing Institute.
- Krauze, M. (2021). *Phytobiotics, a Natural Growth Promoter for Poultry*. In *Veterinary medicine and science*. IntechOpen.
- Li, J., Wu, J., Yu, L., Kong, F., Zhang, R., Sun, J., Liao, W., Li, Z., Shi, J., Wang, Y., Wei, Y., Zhang, K., & Lei, Z. (2022). *Oregano Essential Oils Mediated Intestinal Microbiota and Metabolites and Improved Growth Performance and Intestinal Barrier Function in Sheep*. *Frontiers in Immunology*, 13.
- Makumi, A., Mhone, A. L., Odaba, J., Guantai, L., & Svitek, N. (2021). *Phages for Africa: The Potential Benefit and Challenges of Phage Therapy for the Livestock Sector in Sub-Saharan Africa [Review of Phages for Africa: The Potential Benefit and Challenges of Phage Therapy for the Livestock Sector in Sub-Saharan Africa]*. *Antibiotics*, 10(9), 1085. Multidisciplinary Digital Publishing Institute.
- Molnár, A., Such, N., Farkas, V., Pál, L., Menyhárt, L., Wágner, L., Húsvéth, F., & Duplecz, K. (2020). *Effects of Wheat Bran and Clostridium butyricum Supplementation on Cecal Microbiota, Short-Chain Fatty Acid Concentration, pH and Histomorphometry in Broiler Chickens*. *Animals*, 10(12), 2230.
- Rau, S., Gregg, A., Yaceczko, S., & Limketkai, B. N. (2024). *Prebiotics and Probiotics for Gastrointestinal Disorders*. *Nutrients*, 16(6), 778.
- Revankar, N. A., & Negi, P. S. (2023). *Biotics: An emerging food supplement for health improvement in the era of immune modulation [Review of Biotics: An emerging food supplement for health improvement in the era of immune modulation]*. *Nutrition in Clinical Practice*, 39(2), 311. Wiley.
- Santhiravel, S., Bekhit, A. E. A., Mendis, E., Jacobs, J. L., Dunshea, F. R., Rajapakse, N., & Ponnampalam, E. N. (2022). *The Impact of Plant Phytochemicals on the Gut Microbiota of Humans for a Balanced Life [Review of The Impact of Plant Phytochemicals on the Gut Microbiota of Humans for a Balanced Life]*. *International Journal of Molecular Sciences*, 23(15), 8124. Multidisciplinary Digital Publishing Institute.
- Tarradas, J., Tous, N., Esteve-García, E., & Brufau, J. (2020). *The Control of Intestinal Inflammation: A Major Objective in the Research of Probiotic Strains as Alternatives to Antibiotic Growth Promoters in Poultry [Review of The Control of Intestinal Inflammation: A Major Objective in the Research of Probiotic Strains as Alternatives to Antibiotic Growth Promoters in Poultry]*. *Microorganisms*, 8(2), 148. Multidisciplinary Digital Publishing Institute.
- Wang, J., Deng, L., Chen, M., Che, Y., Li, L., Zhu, L., Chen, G., & Feng, T. (2024). *Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade [Review of Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review*

- of the literature of the last decade]. *Animal Nutrition*, 17, 244. KeAi.
- Abdelli, N., Solà-Oriol, D., & Pérez, J. F. (2021). *Phytogenic Feed Additives in Poultry: Achievements, Prospective and Challenges [Review of Phytogenic Feed Additives in Poultry: Achievements, Prospective and Challenges]*. *Animals*, 11(12), 3471. Multidisciplinary Digital Publishing Institute.
- Aron, R. A. C., Abid, A., Vesa, C. M., Nechifor, A. C., Behl, T., Ghitea, T. C., Munteanu, M., Frățilă, O., Andronie-Cioară, F. L., Toma, M. M., & Bungău, S. (2021). *Recognizing the Benefits of Pre-/Probiotics in Metabolic Syndrome and Type 2 Diabetes Mellitus Considering the Influence of Akkermansia muciniphila as a Key Gut Bacterium [Review of Recognizing the Benefits of Pre-/Probiotics in Metabolic Syndrome and Type 2 Diabetes Mellitus Considering the Influence of Akkermansia muciniphila as a Key Gut Bacterium]*. *Microorganisms*, 9(3), 618. Multidisciplinary Digital Publishing Institute.
- El-Ghany, W. A. A., & Soyadi, Y. A. Y. (2020). *Phytobiotics in Poultry Industry as Growth Promoters, Antimicrobials and Immunomodulators - A Review [Review of Phytobiotics in Poultry Industry as Growth Promoters, Antimicrobials and Immunomodulators - A Review]*. *Journal of World's Poultry Research*, 10(4), 571.
- Hotea, I., Dragomirescu, M., Berbecea, A., & Radulov, I. (2022). *Phytochemicals as Alternatives to Antibiotics in Animal Production. In Veterinary medicine and science. IntechOpen.*
- Konieczka, P., Ferenc, K., Jørgensen, J. N., Hansen, L. H. B., Zabielski, R., Olszewski, J., Gajewski, Z., Mazur-Kuśnerek, M., Szkopek, D., Szyryńska, N., & Lipiński, K. (2023). *Feeding Bacillus-based probiotics to gestating and lactating sows is an efficient method for improving immunity, gut functional status and biofilm formation by probiotic bacteria in piglets at weaning.* *Animal Nutrition*, 13, 361.
- Krauze, M. (2021). *Phytobiotics, a Natural Growth Promoter for Poultry. In Veterinary medicine and science. IntechOpen.*
- Molnár, A., Such, N., Farkas, V., Pál, L., Menyhárt, L., Wágner, L., Húsvéth, F., & Duplecz, K. (2020). *Effects of Wheat Bran and Clostridium butyricum Supplementation on Cecal Microbiota, Short-Chain Fatty Acid Concentration, pH and Histomorphometry in Broiler Chickens.* *Animals*, 10(12), 2230.
- Revankar, N. A., & Negi, P. S. (2023). *Biotics: An emerging food supplement for health improvement in the era of immune modulation [Review of Biotics: An emerging food supplement for health improvement in the era of immune modulation]*. *Nutrition in Clinical Practice*, 39(2), 311. Wiley.
- Tarradas, J., Tous, N., Esteve-García, E., & Brufau, J. (2020). *The Control of Intestinal Inflammation: A Major Objective in the Research of Probiotic Strains as Alternatives to Antibiotic Growth Promoters in Poultry [Review of The Control of Intestinal Inflammation: A Major Objective in the Research of Probiotic Strains as Alternatives to*

Antibiotic Growth Promoters in Poultry].
Microorganisms, 8(2), 148.
Multidisciplinary Digital Publishing
Institute.

Wang, J., Deng, L., Chen, M., Che, Y., Li, L., Zhu,
L., Chen, G., & Feng, T. (2024).
Phytogenic feed additives as natural
antibiotic alternatives in animal health and
production: A review of the literature of the
last decade [Review of Phytogenic feed
additives as natural antibiotic alternatives
in animal health and production: A review
of the literature of the last decade]. *Animal
Nutrition*, 17, 244. KeAi.

