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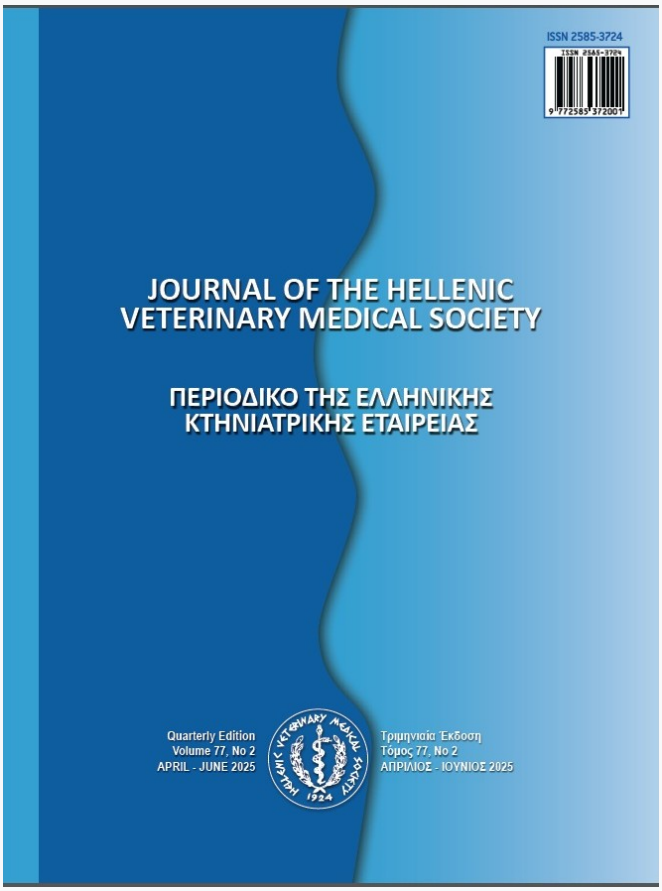

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Research article
Ερευνητικό άρθρο

Effect of dietary inclusion of Sea – buckthorn (*Hippophae rhamnoides*) on health and productivity of mink

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ABSTRACT: The objective of this study was to assess the effect of Sea-buckthorn (SB) on mink health and productivity. The experiment lasted 10 months and was divided into reproductive and growing periods. 100 brown female mink were randomly assigned to two groups (n=50): SB (1%SB) and Con (control). Body weight (BW) of animals during reproduction period was measured. Mating (MR) and whelping rate (WR), litter size (LS) and kit mortality (KM) were recorded. BW of SB group was higher until the end of mating ($p<0.05$). MR, WR, LS, and KM were similar between groups ($p>0.05$), however SB group weaned more kits. Post weaning, 100 offspring from each group (50 males:50 females) were randomly selected and equally allocated to groups: A and B (1%SB) and C and D (control). Groups A and C descended from SB group, while B and D from Con. IL-4, IL-8 and IFN-γ levels in blood serum and Tgf-β1, IL-1B, IFN- γ and IL-6 in spleen samples were determined. Immune cells (B-lymphocytes, granulocytes, T-lymphocytes) in colon, mesenteric lymph node (MLN) and spleen were counted. Blood serum cytokines levels were significantly higher in group B compared to D. Group A had higher IL-4 levels compared to D. The levels of IL-8 and IFN-γ decreased in A and C compared to D. In spleen samples Tgf-β1 was lower in B compared to the rest ($p<0.05$), while IL1-B in A was higher compared to C and D ($p<0.05$). Moreover, IL-6 was higher in group A compared to D ($p<0.01$). A reduction of T-lymphocytes in MLN of A compared to those of B was observed and, in the T-lymphocytes in colon of A compared to the rest groups ($p<0.05$). In spleen, B-lymphocytes were decreased in group A, compared to B and D, as also in C, compared to B and D ($p<0.001$). Microbiome analysis revealed enrichment of *Lactobacillus* in group A and higher abundance of *Candidatus Arthromitus, Clostridium sensu stricto 1*, and *Escherichia-Shigella* in D. The findings suggest that 1% SB affected the gut bacterial flora and the immune system tone of mink, while it presented limited effect on their productive traits.

Keyword: mink; sea-buckthorn; reproduction; microbiome; immune response.

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INTRODUCTION

Mink is, worldwide, one of the most common fur – bearing farmed animals. Female mink breeders are selected based on their reproductive results, while the amount and size of the produced skins determine the profitability of a mink farm (Boudreau et al., 2014; Korhonen & Huuki, 2015). Mink are obligatory carnivores, and their nutritional demands are notably high during their overall breeding period (Jiang et al., 2015; Matthiesen et al., 2010). The growing period in mink lasts approximately 5 months. In order to optimize their performance, relative to their body size in such a short period, an intensive feeding combined with high feed energy content is applied. This combination may result in increased production of reactive oxygen species (ROS) and, in turn, in oxidative stress, challenging the health, productivity and wellbeing of animal livestock (Zhang et al., 2020). Consequently, diminished reproductive traits, metabolic irregularities, and diseases, like fatty liver disease, as well as animal losses may be observed. Therefore, metabolic diseases can exert a significant economic impact on mink farming. Dietary interventions are often a possible way to modulate the excess of ROS and subsequently, to avert or mitigate the oxidative stress (Zhang et al., 2020). For this purpose, numerous feed supplements, particularly antioxidants, are commonly used in mink farming, during both the reproductive and growing periods. Elsewhere, the quest for optimizing the health, as well as, the productivity of fur animals, in a cost – effective way, remains a concern for farmers. The high cost of feed supplements designed exclusively for mink renders the investigation of alternative natural sources, rich in nutritional properties, imperative.

Sea – buckthorn (*Hippophae rhamnoides*) is a plant, that belongs to the *Elaeagnaceae* family, and it is utilized for centuries in human and animal nutrition, owing to its preventive, therapeutic and health promoting effects. Sea buckthorn (SB) possesses unique nutritional properties, as it is a natural reservoir of essential fatty acids, bioactive components, vitamins, carotenoids, phenolic compounds (Ciesarová et al., 2020; Olas, 2016) and proteins (Yuan et al., 2018). Due to its properties, SB is considered a nutraceutical, as it combines both nutritional and pharmaceutical effects (Balkrishna et al., 2019) and it has great potential as a feed supplement for animal livestock.

The primary pharmacological effects of SB are mainly based on antioxidant and anti – inflammatory properties, as the majority of its biological activities are associated with the regulation of antioxidant enzymes and inflammatory cytokines, respectively (Kumar et al., 2011; Ren et al., 2020). Specifically, *in vitro* and *in vivo* studies in rats and hamsters, reveal that SB exhibits significant immunomodulatory properties, as it participates in various pathways, and promotes the activation of cell – mediated immune response, through the regulation of specific cytokines (Mihal et al., 2023; Suryakumar & Gupta, 2011). In the last decades, SB and its compounds have been extensively studied both in human and animals, for their therapeutic effects on skin, gastrointestinal and cardiovascular diseases (Chandra et al., 2018). Recent research revealed that SB affects composition of gut microbiota in human (Chen et al., 2022), mice (Yuan et al., 2018) and hamsters (Hao et al., 2019), and at the same time, it is a good source of prebiotic substrate, as it promotes the proliferation of beneficial gut microbiome (Attri et al., 2018). Furthermore, it is reported that SB enhanced skin health both after oral and topic use (Ren et al., 2020) and it encouraged wound healing in rats and mice, due to its antimicrobial, anti – oxidative and anti – inflammatory properties (Poljšak et al., 2020). In addition, many SB's compounds possess the potential to impact the reproductive system, as evidenced by prior studies, where, their influence on reproductive hormones, inflammatory cytokine levels, and ovarian cell viability have been unveiled (Mihal et al., 2023).

Considering the properties of SB, it was hypothesized that SB could affect the overall health, well – being, and productivity of mink and could constitute a safe and effective feed supplement for the animals. The objective of this study was to investigate the effect of dietary supplementation with SB on reproductive traits of female mink breeders, as also on the growth, immune system, skin quality and intestinal microbiota of growing mink, simultaneously studying the maternal effect of the supplement on the kits.

MATERIALS AND METHODS

Experimental Design

Animals and Housing

Animals involved in the study were raised under standard mink farm conditions, in a mink farm in Kozani prefecture, Northwestern Greece. A total of 300 healthy, free of Aleutian Disease Virus, brown mink (Mustela vison) were used. Animals were

housed in roofed sheds with open sides, in individual standard mink cages with attached wooden nest boxes, which were bedded with straw. The total experimental period lasted ten months (1st of February to end of November 2021) and it was divided into two phases. The study involved dietary inclusion of SB berries in the diet of female breeders and growing mink. To study the effect of SB supplementation on reproduction, 100 brown, yearling, female mink, housed one by one in individual cages, were randomly allocated to two groups: control group (Con group, n = 50), where mink were fed with the basal diet and the Sea – buckthorn group (SB group, n = 50), where the basal diet was supplemented with 1% SB berries. The experiment involved inclusion of SB berries starting 1 month prior to mating season (early February) and lasting for 4.5 months, until weaning of kits, on 14th June (phase 1). Four weeks post – partum kits started to have access to solid feed in addition to milk and were fed the same feed as their dams. After weaning dams were removed from the experiment and then, one female and one male offspring mink were housed in growth cages until their pelting (25 growth cages per treatment).

To study further the effect of SB supplementation on health, growth, and pelt quality of mink, as well as the potential maternal effect of SB supplementation on offspring, a second treatment was conducted, post – weaning (phase 2). In total, 100 offspring (50 females: 50 males) of SB group and 100 offspring (50 females: 50 males) of Con were randomly selected. The animals were equally divided into four groups. Groups A and C descended from SB group. Group A (n = 50, 25 females: 25 males) was daily supplemented with 1% SB berries, until the pelting period, while group C (n = 50, 25 females: 25 males) consisted of mink that were fed the basal diet, without inclusion of SB berries (Figure 1). Respectively, groups B and D descended from the experimental group of Con dams. Group B (n = 50, 25 females: 25 males) consisted of animals that received 1% SB berries daily with their feed until the end of the experiment, while group D (n = 50, 25 females: 25 males) consisted of animals that did not receive SB. The total experimental period of the second experimental phase was 5 months (end of June 2021 to end of November 2021) and it is presented in Figure 1. The whole experiment was performed as a single iteration. The animals were fed a standard, commercial mink diet, supplied daily to the farm by the manufacturer. SB berries were purchased monthly throughout the experimental period, from Hippophae Hellas agricultural cooperative. SB berries were stored in the farm's refrigerator unit. The berries were initially processed in a standard blender and subsequently incorporated into the basal feed of mink. Mink had free access to drinking water from an automatic nipple drinker throughout the experimental period.

Feed and SB berries chemical analysis

A nutrient analysis and total phenolic concentration of feed samples was performed. Six samples of control feed and six with SB were used. The samples of control feed were collected directly from the feed silo before distribution to the feeding machine, while those with SB, were collected from the feed machine, after inclusion of SB. All samples were kept in the freezer of the farm and then they were transported on ice packs to the laboratory. After thawing, the samples were analyzed. The chemical analysis of the feed was carried out at the Laboratory of Animal Nutrition at the School of Veterinary Medicine, Faculty of Health Sciences, of Aristotle University of

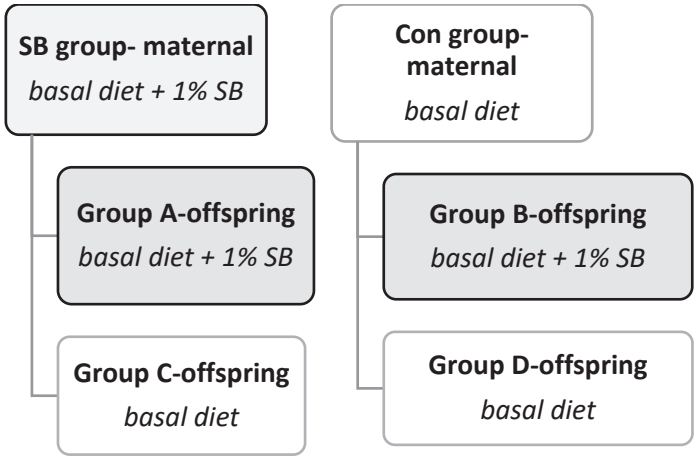

Figure 1. Experimental design: Group A and C descended from female breeders, that were supplemented with *Sea – buckthorn,* during phase 1. Respectively, Group B and D descended from female – control animals. During the second experimental phase (phase 2), Group A and B were treated with *Sea – buckthorn*, while Group C and D, were control animals.

Thessaloniki, using a near-infrared reflectance (NIR) DA7250 Perten Instrument (Perten, Instrumentvägen 31, SE-126 53 Hägersten, Sweden).

Fatty acid composition was determined by Gas Chromatography, according to O'Fallon et al. (2007). The Gas Chromatographic system (Trace-GC model K07332, ThermoFinnigan, ThermoQuest, Milan, Italy) used for methyl esters separation and quantification was equipped with a flame ionization detector, a 5.01 version of Chrom-Card data system (Thermo Electron Corporation, Milan, Italy) and a fused silica capillary column (phase type SP-2380; 30 m × 0.25 mm ID, 0.20 μm film) (Supelco, Bellefonte, PA, USA). The initial oven temperature was 37 °C, held for 4 min, subsequently increased at a rate of 4 °C/min to 250 °C for 5 min. The rest chromatographic conditions were: Inlet temperature: 250 °C, Detector temperature: 260 °C, Injection: 2μl, and Split ratio: 1/50. Fatty acid methyl esters were identified by comparing their retention times and elution order with the Supelco '37 Component FAME Mix' reference standard (Sigma-Aldrich, Darmstadt, Germany). Results were expressed as percentage (%) of the total peak areas for all quantified acids. Fatty acids were classified to saturated, polyunsaturated, and monounsaturated (Giannenas et al., 2016).

The total phenolic content was measured by the Folin–Ciocalteu assay (Lamuela-Raventós, 2017), and the results were measured as mg/ L of gallic acid equivalent (mg GAE/L) extract. In feed samples with inclusion of SB berries, the degree of lipid oxidation was determined after hydrolysis of 1,1,3,3-tetramethoxypropane into malondialdehyde (MDA). The results were expressed as ng MDA/g of the sample. The analysis was performed after the samples were dried (Table 1).

For the chemical analysis of SB berries, determination of proteins, moisture, ash, crude fibers, and fat was performed according to the Weende method, as also of total phenolics, after drying of the samples (Table 2). The crude fiber content in SB berries was determined according to AOAC, Official Method 962.09: Fiber (Crude) in Animal Feed and Pet Food.

Table 2. Chemical analysis of SB berries

Moisture (%)	74.69
Protein (%)	13.14
Fat (%)	21.11
Ash (%)	3.24
Crude fiber (%)	29.79
Total phenolics (mg GAE/L extract)	575.10

Table 1. Chemical analysis and total phenolic content of feed

	Con Group (±SD)	SB Group (±SD)
Moisture (%)	3.7±0.63	3.8±0.37
Protein (%)	37.7±3.13	38.5±3.23
Fat (%)	20.8±2.53	20.5±2.44
Ash (%)	6.0±3.01	5.6±1.63
Total phenolics (mg GAE/L extract)	587.3±32.93	615.9±26.05
Arginine (%)	1.9±0.17	2.0±0.17
Isoleucine (%)	1.0±0.16	1.1±0.21
Leucine (%)	1.6±0.35	1.6±0.52
Lysine (%)	1.9±0.28	2.0±0.25
Methionine (%)	0.9±0.18	1.0±0.25
Threonine (%)	1.0±0.07	1.0±0.17
Tryptophan (%)	0.3±0.04	0.3±0.38
Valine (%)	0.8±0.25	0.7±0.37
MDA (ng MDA/g sample)	466.0±126.82	438.3±98.54
Saturated fatty acid (%)	39.0±7.70	35.3±3.64
Polyunsaturated fatty acid (%)	24.7±4.17	24.4±3.40
Monounsaturated fatty acid (%)	36.3±9.52	40.3±1.38

Reproductive Performance

Mating of female mink started on March 3rd and lasted until 15th March 2021. All the breeding females were yearlings and animals mated according to the normal mating system 1 + 8 + 1 (Felska-Błaszczyk et al., 2019). For the assessment of SB effect on reproductive performance of female mink, date of each mating, date of parturition and litter size were recorded. During whelping period all females were daily monitored for evidence of whelping. For the calculation of litter size and mortality, kits were counted at birth, two – and ten – days post – partum and at weaning. After that, pre – weaning mortality was calculated between birth and weaning (14 June).

Values for the reproductive performance of female mink were analyzed: mated females (percentage of mated females that gave birth), barren females (females that mated at least once, but they did not have parturition sign), litter size at birth, day 2 (d2), day 10 (d10) and at weaning, mortality of kits (percentage of mortality of kits during maternal nursing). Unmated and empty female mink were removed from the experiment directly after mating and whelping period, respectively.

Body weight and Body length

Initial body weight of female breeders was individually measured at the beginning of the experiment (BWb) and it was 1412.8 (±152.98) grams for Con group and 1350.4 (±157.76) grams for SB group. A second measurement was performed in late March, after mating and at the first gestation period (BWm). A third measurement was performed during lactation period, in late May (BWl). The final body weight of dams was measured at weaning of kits, in the middle of June (BWw).

To study the effect of SB on growth of kits during the second experimental phase, the body weight of 200 growing mink (25 females and 25 males of each group) was individually measured once per month from weaning to 2 weeks before pelting, except from month August, due to practical reasons. In total, four measurements of the offspring's body weight were conducted. Body weight differences between consecutive measurements were calculated.

At last, the body length of mink was measured before pelting. Animals were placed in a graded plastic tube and body length from the nose to tail was recorded.

Hair re-growth assessment, Live grading and Fur quality scoring

For hair re – growth assessment, 10 mink (5 females, 5 males) from groups B and D respectively, were used. A square area of dimension 5 x 5 cm in the caudal ventral back area was shaved using a normal electric clipper and was photographed every 3 days until the 42nd day post-shaving (15 shootings). Next, a subjective evaluation of the images was performed by 2 expert fur quality evaluators using a 0 – 5 scale in each time – point.

Before pelting, live grading of skins was performed in the farm by skilled professional pelt graders, in a scale of 1 – 5 (1 lowest quality, 5 best quality), according to the following criteria: density of underfur, body length, pelt nap length and the overall impression of the fur.

Drying of skins was performed in a local drying center in Greece and after that, skins were delivered to Saga Furs Auction House in Finland, where they were graded and sold in an auction. Size of pelt, size of nap, overall quality of skins and price were recorded. Categorization and scoring of skins were performed according to Saga Furs standards.

Euthanasia and Necropsy

Mink were euthanized using a single killing box with carbon monoxide gas by an experienced and certified individual, following European standards. A full necropsy was then performed, and tissue samples were collected for pathology evaluation, cytokine level determination, and microbiome analysis, as described below.

Histopathology, Immunohistochemistry and Morphometry

For comprehensive pathology evaluation, including histopathology, immunohistochemistry, and histomorphometry, tissue samples of colon, skin, mesenteric lymph node (MLN), and spleen (n = 24; 3 females and 3 males from each of the four experimental groups) were collected during necropsy from standardized anatomical sites, fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 4 – 5 μm. Skin thickness was evaluated in HE – stained skin sections from 12 randomly selected animals. Additionally, quantitative assessment of IHC – stained immune cells, using primary antibodies against MPO for granulocytes (ThermoFisher Scientific/Lab Vision, Fremont, CA, USA), PAX5 for B – lymphocytes (Abcam, Cambridge, UK), and CD3 for T – lymphocytes (Cell Marque, Rocklin,

CA, USA) was performed in colon, MLN, and spleen sections from six randomly selected animals. Both evaluations followed the methodology previously described by our group (Iatrou et al., 2023).

Cytokines Profiling Assay

Quantitative Gene Expression Analysis

For the quantitative gene expression of Transforming Growth Factor beta – 1 (Tgf – β1), Interleukin – 1B (IL – 1B), interferon – γ (IFN – γ) and Interleukin – 6 (IL – 6) total RNA was extracted from spleen tissue samples (n = 24, 3 females: 3 males for each of all the four experimental groups). Methods and conditions of the analysis have been previously described (Iatrou et al., 2023). The real time PCR thermal cycler used in current study was SaCycle-96 real time PCR system (Sacace Biotechnologies Srl, Como, Italy).

Cytokines Detection

Interleukin – 4 (IL – 4), interleukin – 8 (IL – 8) and interferon – γ (IFN – γ) levels were determined in mink blood serum (n = 24, 3 females: 3 males for each of all the four experimental groups) by an in – house sandwich enzyme – linked immunosorbent assay (ELISA). To select a suitable reagent as the blocking buffer for the ELISA, a comparative test for IL – 4 and IFN – γ was carried out using four different solutions, namely: Synblock buffer (BUF034, Bio-Rad Laboratories, Inc., Hercules, CA, US), 5% skimmed milk, 1% Bovine Serum Albumin, and 5% Tween – 20. The sandwich ELISAs were performed according to the protocol described by Cronin et al. (2015) with some modifications. In more detail, to bind the associated serum antigens, 96 – well plates were coated with the appropriate monoclonal antibody in 1X ELISA Coating Buffer (BUF030, Bio-Rad Laboratories, Inc., Hercules, CA, US) and incubated at room temperature overnight (Table 3). After one hour of incubation with the blocking solution, samples and standards were added and incubated at room temperature for one and a half hours. Then, the appropriate polyclonal antibodies were added, and the plates were incubated for 2 hours at room temperature, followed by a one-hour incubation with the Horseradish peroxidase-conjugated goat anti-rabbit IgG antibody. After the addition of colorimetric substrate 3,3',5,5' – tetramethylbenzidine TMB Core+ (BUF062A, Bio-Rad Laboratories, Inc., Hercules, CA, US) and incubating in the dark for half an hour, the reaction was terminated by adding a solution of 0.5 M sulfuric acid (H_2SO_4). Intermediate washes were carried out in triplicate using 150 μL of 1X ELISA Wash Buffer (BUF031, Bio-Rad Laboratories, Inc., Hercules, CA, US). Optical absorbance measurements were taken at 450 and 550 nm using the Spark® 20M multimode reader system (Tecan Group Ltd., Männedorf, Switzerland). The blocking solution was utilized as a negative control, while a mink serum sample positive for the cytokines in question served as a positive control. All reactions were carried out in duplicate.

To account for 96 – well plate biases, optical absorbance values at 550 nm were subtracted from those at 450 nm for statistical analysis. The average values derived from the two replicates were calculated, and the negative control values were subtracted from the sample values.

Bacterial Flora Microbiome Analysis

For the assessment of the intestinal bacterial microbiome, fecal samples (n = 24, 3 females: 3 males for each of all the four experimental groups) of mink were collected; library construction, sequencing and bioinformatics analysis have been previously described (Iatrou et al., 2023).

Table 3. ELISA immunoassay antibodies and concentrations

Antibody	IL-4 assay	IL-8 assay	IFN-γ assay	Concentration
Primary Antibody	Monoclonal Mouse anti-Bovine IL-4 antibody, clone CC303	Monoclonal Mouse anti-Sheep IL-8 antibody, clone 2C9/8M6	Monoclonal Mouse anti-Bovine IFN-γ antibody, clone CC302	2.5 μg/mL
Secondary Antibody	Polyclonal Rabbit anti-Rat IL-4 antibody	Polyclonal Rabbit anti-Sheep IL-8 antibody	Polyclonal Rabbit anti-Ferret IFN-γ antibody	0.2 μg/mL
Tertiary Antibody	Polyclonal Goat anti-Rabbit IgG (H&L) antibody HRP	Polyclonal Goat anti-Rabbit IgG (H&L) antibody HRP	Polyclonal Goat anti-Rabbit IgG (H&L) antibody HRP	1:10,000

Statistical Analyses

A Kolmogorov – Smirnov test of normality was used to verify the distribution of variables for each measurement. Pairwise comparisons of categorical variables, including skin quality, grading on alive animals, size and nap of pelts were conducted using a Chi - square independence test. To compare the skin price between the experimental groups, as well as the values for body weight and body weight difference, a univariate analysis of variance (ANOVA) was performed. Treatment group was used as a fixed factor, while each statistical analysis performed per sex to all the above analyses. To determine the statistical significance of the results in cytokines ELISA analysis, an unpaired t – test was performed. A Mann – Whitney U analysis was used for histomorphometric counts and hair re – growth assessment analysis.

For the analysis of hair re – growth assessment, grading on alive animals, skin quality, size and nap of pelts and skin price the SPSS v. 25.0 software (IBM Corp., Chicago, IL, USA) was used. For all the rest analyses and the visualization of the results, the Graphpad Prism (version 9.1.2 for Windows®, GraphPad Software, San Diego, CA, USA) was utilized. Data representation was done with bar graphs depicting the mean and standard error of the parameter assessed for each experimental group. Statistical significance was set at $p < 0.05$, whereas values between $0.05 < p < 0.10$ were considered as a "trend".

RESULTS

Histopathology, Immunohistochemistry, Morphometry

Regarding skin thickness no statistically significant difference was detected in either male or female animals, separately or combined, across the experimental groups ($p > 0.05$) (Figure 2).

Concerning the effect of SB berries on the immune system of mink, a reduction of CD3+ T – lymphocytes in the colon was observed in animals of group A compared to the other experimental groups (A vs. B, $p < 0.001$; A vs. C, $p = 0.001$; A vs. D, $p = 0.018$). However, both B – lymphocytes (PAX5+) and granulocytes (MPO+) showed comparable values across all groups ($p > 0.05$) (Figure 3). Similarly, in the MLN tissue, CD3+ T – lymphocytes were decreased in group A compared to group B ($p = 0.04$), while all other values showed no statistically significant differences ($p > 0.05$) (Figure 4). In the spleen, SB berries supplementation resulted in a significant reduction in PAX5+ B – lymphocytes in groups A and C compared to groups B and D (A vs. B, $p < 0.001$; A vs. D, $p < 0.001$; C vs. B, $p < 0.001$; C vs. D, $p < 0.001$). In contrast, granulocytes (MPO+) and T – lymphocytes (CD3+) had comparable values across all experimental groups ($p > 0.05$) (Figure 5).

Cytokines Profiling Assay

Cytokines Detection

Different blocking buffers were tested in order to select the solution with the least non-specific signal in the ELISA experiment. Synblock buffer is a commercially available ELISA assay solution, whereas skimmed milk, bovine serum albumin, and Tween-20 solutions are all frequently used in ELISAs. Negative controls (BLANK) with Synblock solution and 5% skim milk had the lowest optical absorbance readings (0.06), while solutions containing 1% bovine serum albumin and 5% Tween – 20 had significantly higher values (from 0.14 to 1.00) (Figure 6). However, when tested with the Synblock buffer, the optical absorbance values of the mink blood serum samples were similar to the negative control. Thus, for subsequent ELISA assays, 5% skimmed milk

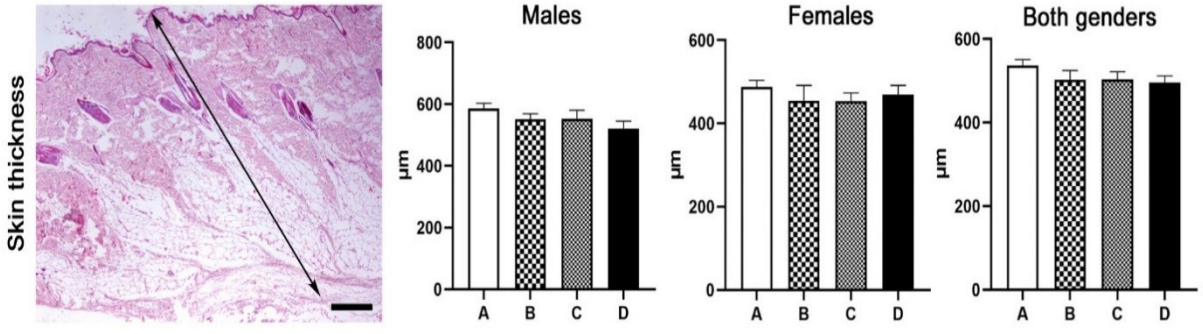

Figure 2. Skin thickness measurements (double – headed arrow) for the four experimental groups. Both female and male mink presented comparable skin thickness, irrespective of the trial diet. Letters of x – axes correspond to the experimental groups (A, B, C, D). Scale Bar: 500μm.

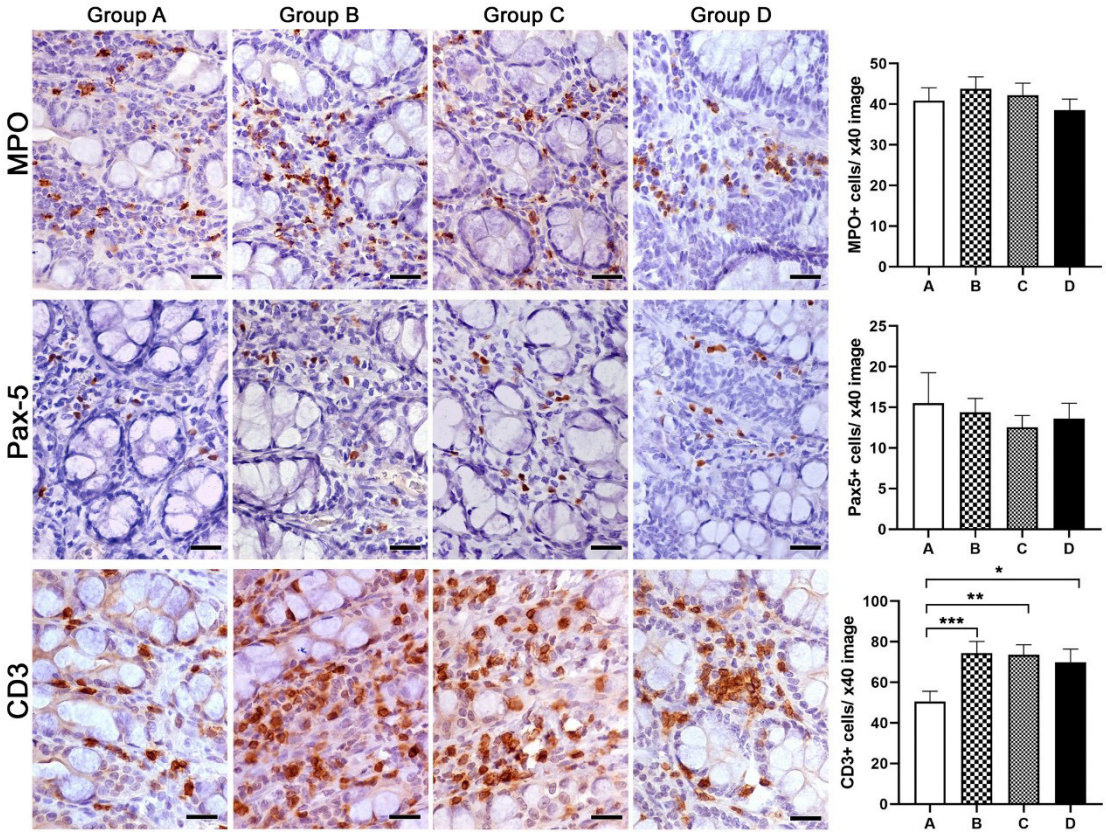

Figure 3. Effects of SB berries supplementation on inflammatory cells in the colonic mucosa for the four experimental groups (A, B, C, D). A reduction of CD3+ T – lymphocytes was observed in animals of group A compared to the remaining experimental groups. IHC: diaminobenzidine chromogen, haematoxylin counterstain. Scale bars: 25 μm. Numbers on the y – axes of bar graphs correspond to the means ± SEM of immune cell counts * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Letters of the x – axes correspond to the experimental groups (A, B, C, D).

was chosen as the blocking solution and antibody dilution solution.

In the different SB dietary supplementation groups, cytokine levels in mink blood serum were compared. Groups B and D were found to be statistically significantly different; Group B samples had the highest levels of IL – 4, with an average optical absorbance of 0.68 (median 0.62), while group D had the lowest, with an average of 0.27 (median 0.15) (Figure 7). A statistically significant difference was also found between groups B and C. Finally, IL – 4 levels in groups A and C were 0.37 and 0.29 on average (median 0.32 and 0.26, respectively).

The highest levels of IL – 8 were detected in group B samples, with a mean of 0.93 (median 0.81), followed by group D samples, with a mean of 0.74 (median 0.66) (Figure 7). IL – 8 levels were 0.55 and 0.62 in groups A and C, respectively (median 0.50 and 0.62). In addition, there was a statistically significant difference between groups B and D.

Finally, the samples from group B had the highest levels of IFN – γ with a mean of 0.39 (median 0.23), followed by group D with a mean of 0.24 (median 0.18) (Figure 7). IFN – γ levels were 0.15 and 0.13 in groups A and C, respectively (median 0.11 and 0.10). Similarly, groups B and D showed a statistically significant difference in IFN – γ levels.

Quantitative Assessment of Cytokines

Tgf – β1 was lower in group B compared to the rest groups (B vs. A, $p = 0.007$; B vs. C, $p = 0.016$; and B vs. D, $p = 0.001$). IL1 – B in group A was significantly higher compared to group C and D (A vs C, $p = 0.037$; A vs D, $p = 0.009$). The same pattern was also found in IL – 6 cytokine, which was higher in group A compared to D ($p = 0.009$). Regarding IFN – γ, comparable values were found among the groups ($p > 0.05$) (Figure 8).

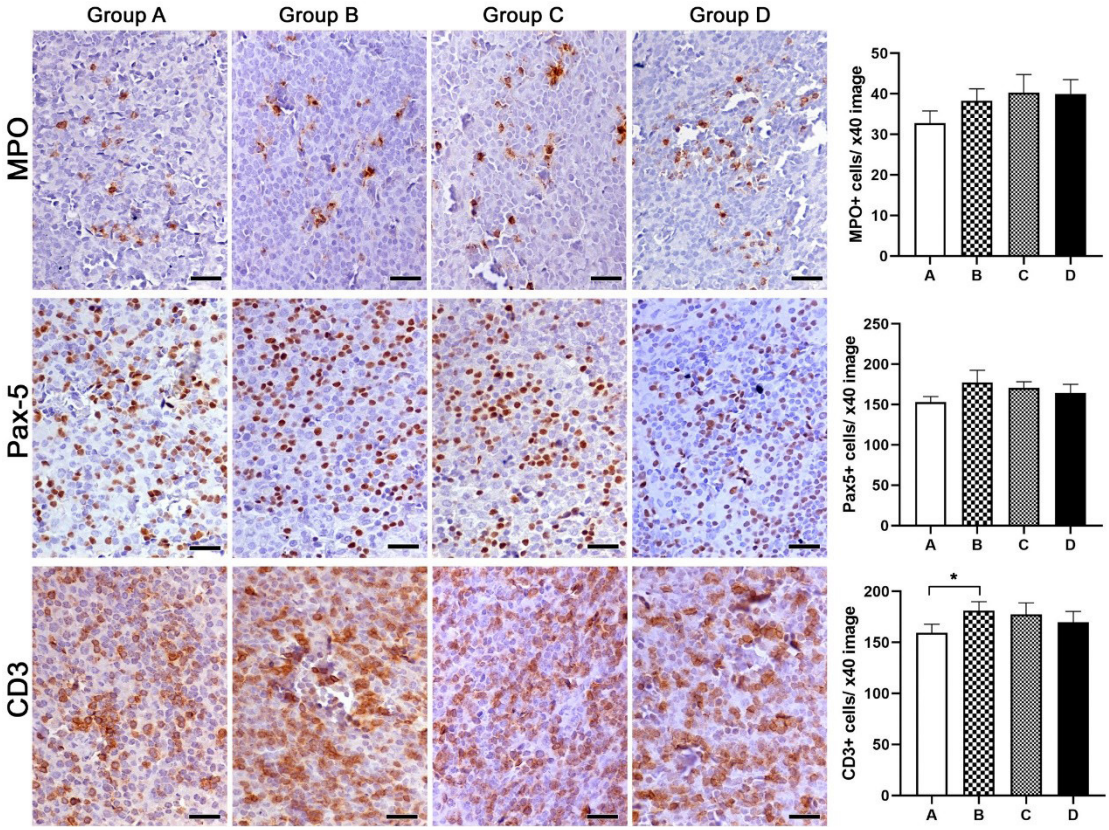

Figure 4. Effects of SB berries supplementation on inflammatory cells in paracortical areas of MLN for the four experimental groups (A, B, C, D). A reduction of CD3+ T – lymphocytes was observed in animals of group A compared to those of group B. Numbers on the y – axes of bar graphs correspond to the means ± SEM of immune cell counts *: $p < 0,05$. IHC: diaminobenzidine chromogen, hematoxylin counterstain. Scale bars: 25μm. Letters of the x – axes correspond to the experimental groups (A, B, C, D).

Bacterial Flora Microbiome Analysis

A total of 13,183,207 paired – end raw sequence reads were generated, resulting in 6,568,092 reads and 1,386 unique ASVs after filtering, denoising and dereplication. Firmicutes was the most abundant Phylum in all groups, averaging 81.1% total relative abundance (Figure 9), followed by Proteobacteria (14.7% of total relative abundance). *Peptostreptococcales, Tissierellales* and *Clostridiales* were prevalent in groups B (av. 92.8%) and D (av. 78.3%), whereas *Lactobacillales* were enriched in group A but remained below 4% in groups B and D. At the family level, *Clostridiaceae* (31.1%), *Peptostreptococcaceae* (25.8%) and *Enterobacteriaceae* (10%) were the most abundant bacteria. Specifically, *Clostridiaceae* (55.8%) was the most abundant family in group D, while in groups B and C *Peptostreptococcaceae* was the most abundant (av. 51.2% and 21.1%, respectively). In group A, *Enterobacteriaceae* (11.1%) and *Lactobacillaceae* (20.1%) were the dominant families. At the genus taxonomic level, the most abundant genera in all groups were *Romboutsia* (22.8%), *Candidatus Arthromitus* (19.1%), *Clostridium sensu stricto 1* (12%), *Escherichia-Shigella* (9.9%) and *Lactobacillus* (7.7%) (Figure 9). Bacteria of the *Romboutsia* genus, particularly *Romboutsia ilealis,* were mainly detected in groups B, C and D. *Lactobacillus* was detected in group A (mainly *Lactobacillus panis* and *L. aviarius* species) and in decreased levels in group C, while it was absent from groups B and D. At the species level, bacterial communities were comprised mostly of uncultured bacteria (> 40% in groups B, C and D, and 23% in group A). *Shigella dysenteriae* species was mainly detected in samples of group D with relative abundance ranging from 11.9% to 33.3%and in lower abundance in group A (av. 10.9, B (av. 2.7%), and C (7.2%, 34.3% and 0.4%).

The effect of dietary supplementation of SB ber-

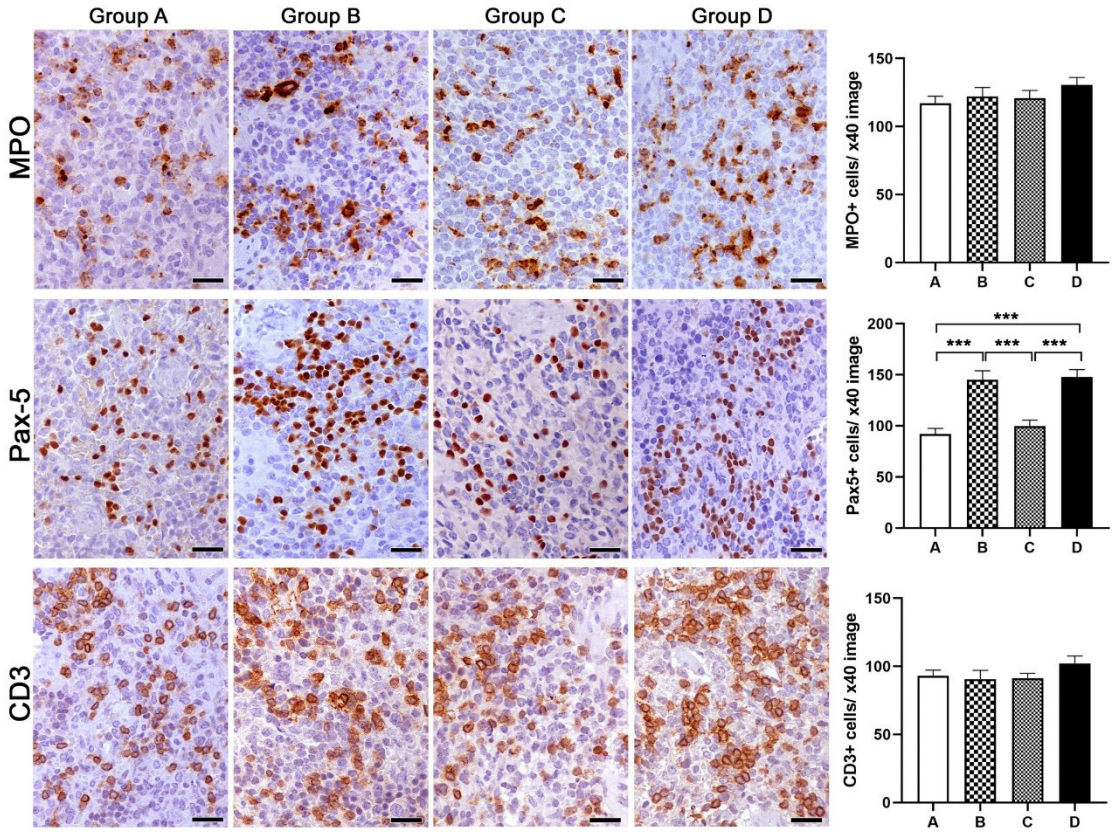

Figure 5. Effects of SB berries supplementation on inflammatory cells in the non-follicular areas of spleen for the four experimental groups (A, B, C, D). Morphometric counts of immunohistochemically labeled B – lymphocytes showed statistically important differences, as highlighted in the bar graphs. IHC: diaminobenzidine chromogen, hematoxylin counterstain. Scale bars: 25μm. Numbers on the y-axes of bar graphs correspond to the means ± SEM of immune cell counts *: $p < 0,05$, **: $p < 0,01$, ***: $p < 0,001$. Letters of the x – axes correspond to the experimental groups (A, B, C, D).

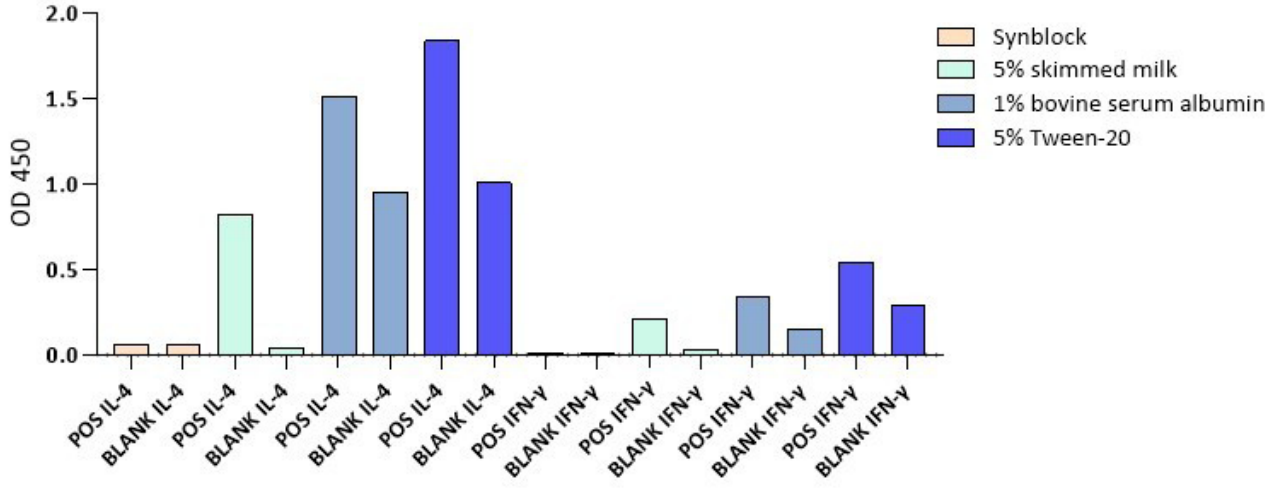

Figure 6. Barplot of optical absorbance values using different blocking solutions: POS: mink blood serum sample, BLANK: negative control; Synblock (beige), 5% skim milk (turquoise), 1% bovine serum albumin solution (light blue), 5% Tween-20 solution (blue).

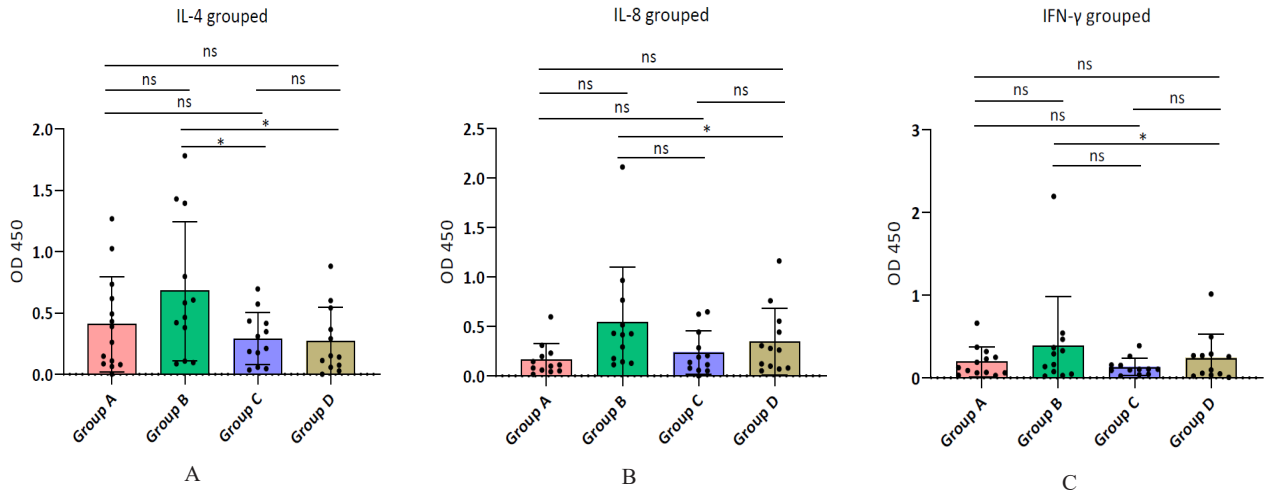

Figure 7. Comparison of optical absorbance values between groups of samples after adjustment with the negative control value (blank-corrected OD 450 – 550). (A) Positive control: mink blood serum sample positive for IL – 4. (B) Positive control: mink blood serum sample positive for IL – 8. (C) Positive control: mink blood serum sample positive for IFN – γ. *$p \leq 0.05$, ns: statistically not significant.

ries was further investigated by testing for differential abundances among the four experimental groups. DESeq2 pairwise comparisons between groups A, B, C, and control group D revealed 36, 31 and 22 ASVs, respectively, with adjusted p – value < 0,01 (Figure 10). Group D had significantly enriched *Clostridiaceae* and *Peptostreptococcaceae* ASVs compared to groups A and C, while group B showed higher *Peptostreptococcaceae* abundance than group D. Group A had 25 enriched ASVs compared to group D, mostly Lactobacillales, followed by *Ruminococcaceae*. Respectively, group C had 10 enriched ASVs compared to group D (mainly *Lachnospiraceae*, *Caulobacteraceae* and *Sphingomonadaceae* species). Comparisons of A and C versus B showed 12 and 21 ASVs, respectively, mostly *Peptostreptococcaceae* (*Romboutsia ilealis*). Group A showed statistically significantly higher abundance of *Lactobacillaceae*, *Fusobacteriaceae*, *Enterococcaceae* and others compared to B, while C had higher *Peptostreptococcales-Tissierellales Sphingomonadaceae* and others than group B. Regarding groups A and C, one ASV of *Rhizobiaceae* was significantly enriched in C, while five ASVs were significantly higher in group A (*Fusobacteriaceae*, *Microbacteriaceae*, *Enterococcaceae* and others).

Reproductive Performance

According to the records collected during the reproductive period, no significant difference was

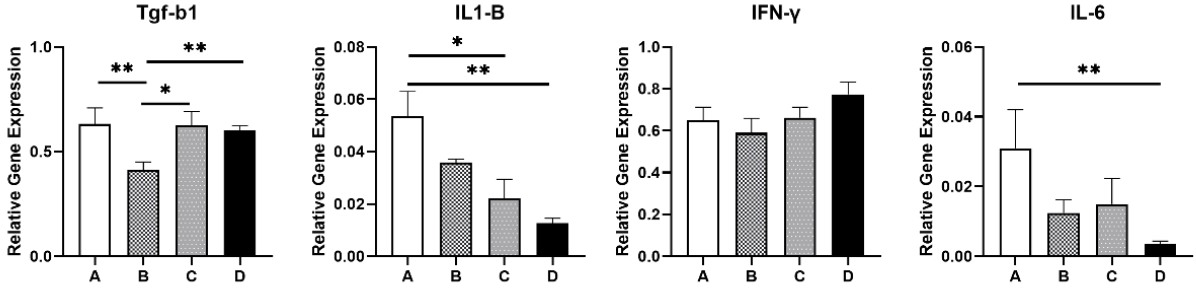

Figure 8. Expression of Tgf – β1, IL1 – B, IFN – γ and IL – 6 in the spleen for the four experimental groups. Statistically significant differences were observed for Tgf – β1, IL1 – B and IL – 6 were observed, as they are highlighted in the bar graphs. *: $p < 0,05$, **: $p < 0,01$. Letters of the x- axes correspond to the experimental groups (A, B, C, D).

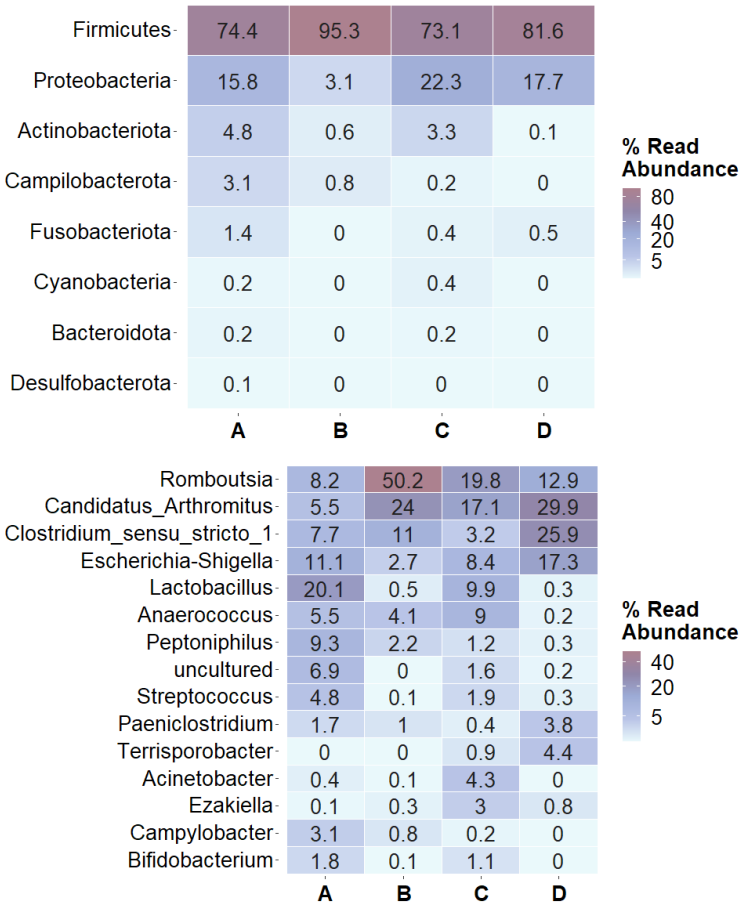

Figure 9. Heatmaps depicting the relative abundances of the most enriched bacteria at the taxonomic level of phylum (left) and genus (right). Numbers reflect the average relative abundance (%) for each diet treatment (groups A, B, C, D).

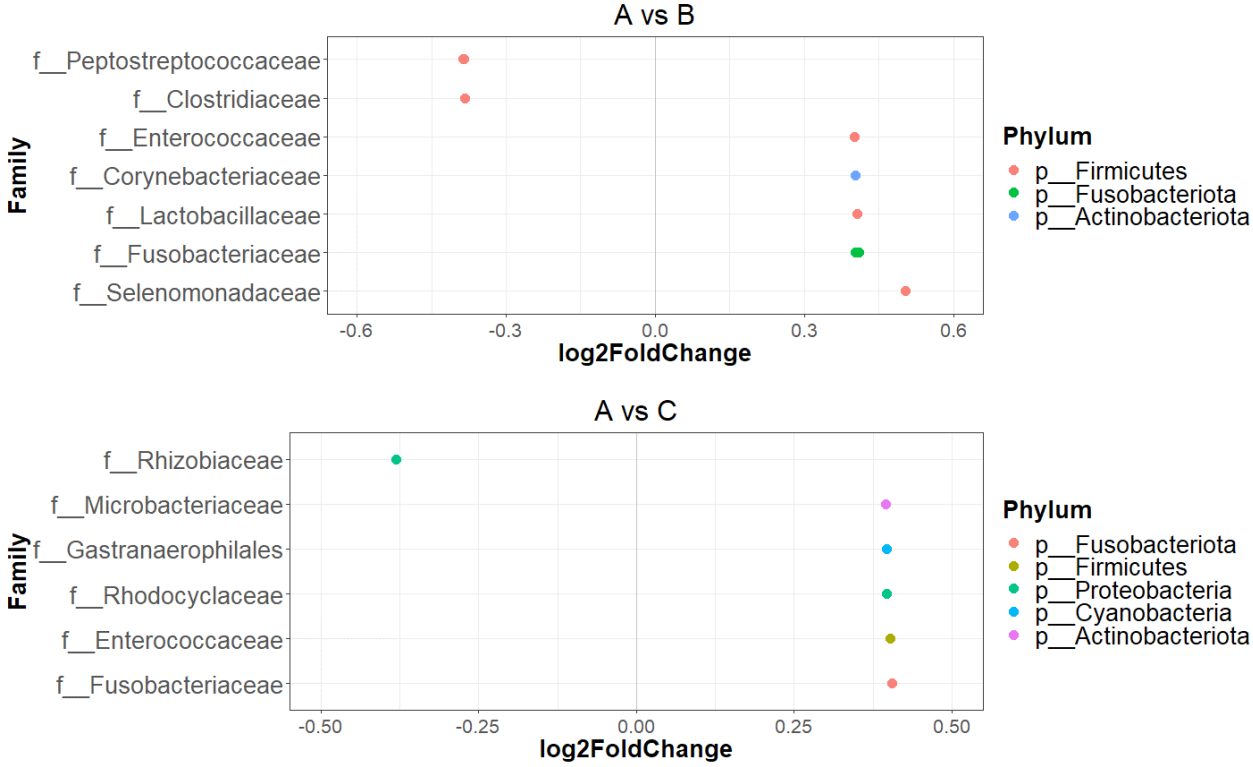

Figure 10.

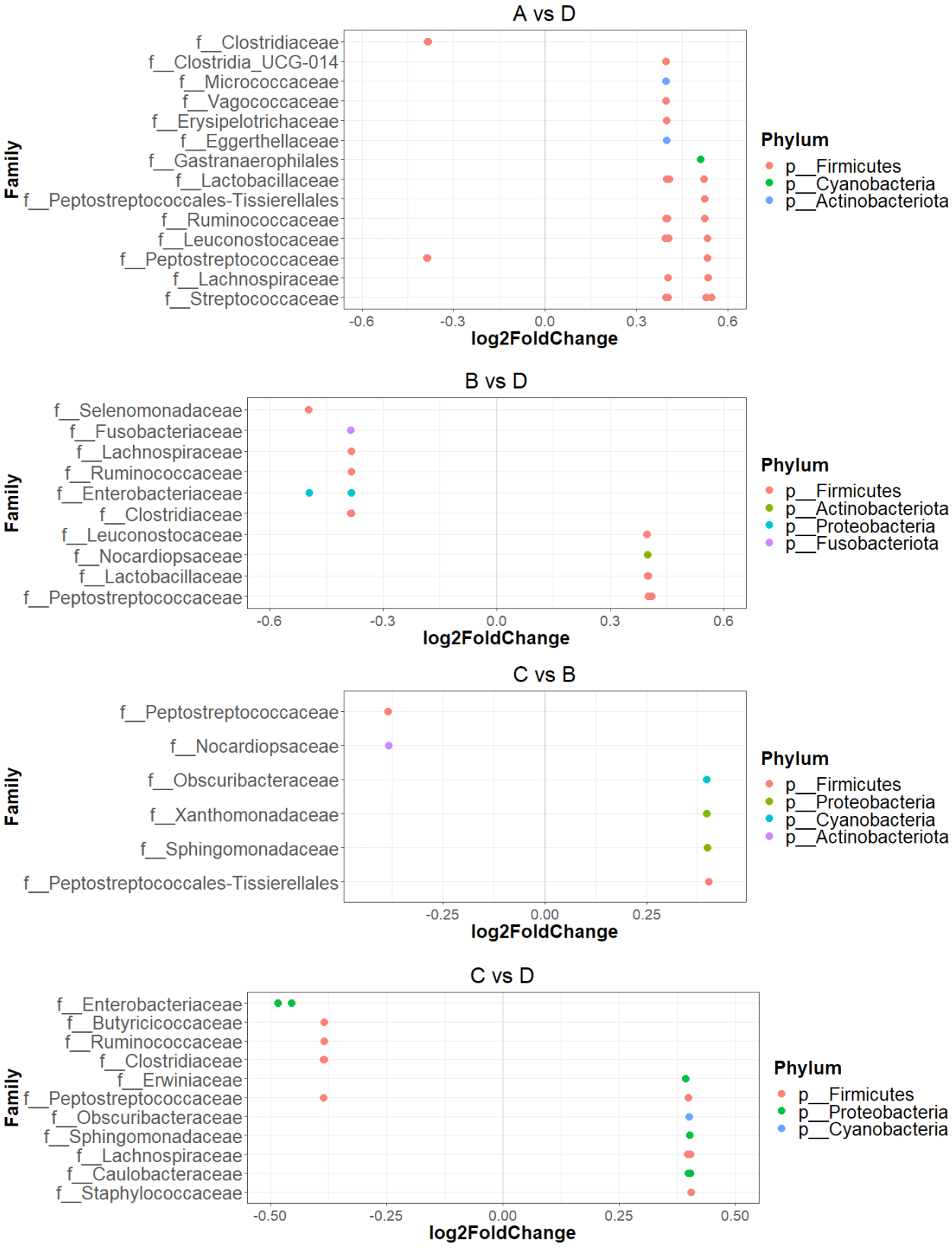

Figure 10. Differentially abundant ASVs depict the log2fold differential abundance of the different families for pairwise comparisons among groups (DESeq2). Adjusted p – value <0.01. Log2(fc) values greater than zero indicate higher abundances in the relevant ASVs of the first group, while negative values indicate an increased abundance in the second group.

observed between the two groups for all the surveyed traits (Supplementary table S1). However, the number of kits weaned by the SB group was finally higher compared to Con group.

Growth, Skin Grading, Hair re – Growth Assessment

The initial BW of the two groups at the first experimental period (phase 1) presented a statistically important difference ($p = 0.043$) and it was considered as a co – variate in the next calculations. During the mating period (BW2 – BW1) a higher BW gain was observed for SB group compared to the Con. However, no statistically significant difference was observed either on BW or on BW gain during the rest of the experimental period (Supplementary table S2).

The BW and BW gain of male and female animals exhibited various fluctuations throughout the experimental period (Supplementary table S3 and S4). While the final BW was similar across the four experimental groups of female animals, differences were observed in that of males, where final BW of group B was lower compared to group C and D (B vs. C, $p = 0.063$; B vs. D, $p = 0.034$).

Regarding body length of female mink, comparable values were observed between the groups ($p < 0.05$). Average body length for group A was 42.30 cm, for group B was 42.24 cm, for group C was 41.88 cm and for group D was 41.92 cm. As far as the body length of male mink is concerned, animals of group C had an increased body length compared to those of group A ($p = 0.030$) and B ($p = 0.018$). Specifically, average body length for group A was 50.63 cm, for group B was 49.36 cm, for group C was 50.87 cm and for group D was 50.88 cm.

SB supplementation did not affect fur re-growth during the whole trial period in mink ($p > 0.05$). The view of the shaved area bore resemblance in both groups, at the end of the experiment.

Table S1. Descriptive statistics (Mean and Standard Deviation) for Litter Size (LS) of SB group (N = 42) and Con group (N = 40), at birth, two (d2) and ten (d10) days post – partum and at weaning, as also mortality of kits and number of kits at weaning

	Group		
Timepoint	**SB (±SD[1])**	**Con (±SD[1])**	***p* - Value**
LS[2] birth	8.3 (0.82)	7.9 (1.42)	0.112
LS[2] d2	7.3 (0.82)	6.9 (1.42)	0.109
LS[2] d10	6.4 (1.43)	6.1 (1.25)	0.313
LS[2] weaning	6.1 (1.49)	5.8 (1.39)	0.354
Mortality of kits	2.2 (2.5)	2.2 (1.4)	0.809
Total weaned kits	304	231	

[1] SD= Standard Deviation, [2] LS= Litter Size

Table S2. Descriptive statistics (Mean and Standard Deviation) for body weight in grams of SB and Con groups at the beginning of the experiment (BWb), after mating (BWm), after lactation (BWl) and at weaning (BWw), as also body weight difference between the above intervals

	Group		
Timepoint	**SB (±SD[1])**	**Con (±SD[1])**	***p* - Value**
BWb	1350.4 (151.76)	1412.8 (152.98)	0.043
BWm	1424.4 (148.16)	1443.8 (159.06)	0.173
BWl	1465.0 (144.48)	1488.9 (185.65)	0.863
BWw	1276.2 (138.19)	1299.1 (172.95)	0.119
BWm- BWb	+74.0 (94.46)	+31.0 (117.46)	0.046
BWl- BWm	+15.7 (157.08)	+59.4 (126.42)	0.150
BWw- BWl	-188.8 (125.19)	-189.8 (127.38)	0.831

[1] SD= Standard Deviation

Table S3. Descriptive statistics (Mean and Standard Deviation) for body weight in grams, of female mink for the total experimental period (n = 25 per group)

	Group				
Timepoint	**A (±SD[1])**	**B (±SD[1])**	**C (±SD[1])**	**D (±SD[1])**	***p* - Value**
BW1	924.56 (72.00)	928.00 (95.96)	889.12 (92.32)	898.64 (80.75)	0.297
BW2	1312.72 (102.73)	1274.80 (117.98)	1253.60 (116.46)	1237.68 (88.71)	0.083
BW3	1898.33 (143.58)	1814.80 (184.55)	1764.00 (166.43)	1803.60 (173.01)	0.046
BW4	1995.22 (155.21)	1934.00 (225.20)	1876.80 (259.66)	1902.00 (197.69)	0.260
BW2- BW1	388.16 (64.61)	346.80 (60.93)	364.48 (65.98)	339.04 (54.66)	0.027
BW3- BW2	581.92 (88.34)	540.00 (108.43)	510.40 (83.67)	565.92 (111.20)	0.066
BW4- BW3	100.00 (84.42)	119.20 (84.55)	112.80 (204.48)	98.40 (89.99)	0.927

[1] SD= Standard Deviation

Table S4. Descriptive statistics (Mean and Standard Deviation) for body weight in grams, of male mink for the total experimental period (n = 25 per group)

	Group				
Timepoint	**A (±SD[1])**	**B (±SD[1])**	**C (±SD[1])**	**D (±SD[1])**	***p* - Value**
BW1	1120.6 (102.53)	1128.4 (172.01)	1174.0 (105.92)	1166.9 (161.90)	0.429
BW2	1785.1 (184.20)	1743.7 (224.18)	1871.0 (150.32)	1804.8 (221.49)	0.151
BW3	3183.3 (241.19)	3043.6 (368.63)	3225.4 (328.96)	3292.4 (365.19)	0.078
BW4	3603.3 (324.41)	3418.6 (452.24)	3647.8 (417.86)	3676.0 (428.55)	0.148
BW2- BW1	664.5 (113.04)	615.4 (78.14)	697.0 (69.84)	637.9 (92.94)	0.012
BW3- BW2	1398.2 (149.57)	1310.5 (215.06)	1349.1 (242.38)	1487.6 (177.48)	0.018
BW4- BW3	420.0 (168.96)	375.0 (155.40)	400.4 (135.16)	383.6 (205.71)	0.809

[1] SD= Standard Deviation

Regarding grading of fur on alive mink, both female and male animals presented not statistically significant difference between the four groups (Supplementary table S5). Nap size was similar for female mink ($p = 0.392$). However, male mink of group A presented higher nap size compared to the rest groups ($p = 0.019$). No difference was presented in skin size, both for male and female animals ($p > 0.05$). Final skin quality was similar for all the animals ($p > 0.05$). Skin price of male animals was comparable between the four groups. However, skin price of group A in female animals was higher in group A ($p < 0.01$) compared to the rest groups.

Table S5. Descriptive statistics (Mean and Standard Deviation) for the skins of female and male mink

	Group							
	female	**male**	**female**	**male**	**female**	**male**	**female**	**male**
Trait	**A (±SD[1])**		**B (±SD[1])**		**C (±SD[1])**		**D (±SD[1])**	
Grading	4.0(0.6)	4.0(0.6)	3.7(0.8)	4.0(1.1)	3.5(0.6)	3.3(1.0)	3.3(1.2)	3.8(1.0)
Skin size	1.2(0.4)	3.0(1.1)	1.5(0.6)	2.2(1.2)	2.2(1.0)	3.0(0.9)	2.2(0.8)	3.5(1.0)
Nap size	1.3(0.5)	2.3(0.5)[a]	2.0(0.6)	1.8(0.4)[b]	1.5(0.6)	2.2(0.8)[b]	1.7(0.5)	1.2(0.4)[b]
Skin quality	1.5(0.5)	1.7(0.5)	3.0(0.9)	2.5(0.8)	2.0(0.9)	2.5(0.8)	1.8(0.8)	1.7(0.8)
Skin price (€)	23.8(1.3)[a]	33.1(3.7)	19.7(2.7)[b]	35.(3.7)	20.3(3.8)[b]	32.7(4.5)	20.3(3.0)[b]	36.0(5.0)

[1] SD = Standard Deviation, [a, b] = The values with different exponents were found to exhibit a statistically significant difference at $p < 0.05$

DISCUSSION

In the present study, the effect of SB berries supplementation on health and productivity of mink was investigated.

The intestinal microbiome is tightly connected to the immune system (Ruff et al., 2020) and interacts with remote organs like the brain and skin (Arck et al., 2010). Moreover, according to previous reports, both the composition and the function of the microbial communities in the gastrointestinal tract are impacted by the supplemented diet (Andresen et al., 2015;). Exploiting this occurrence, many feed supplements are used in animal production, to improve the intestinal microflora and in turn, to enhance the gut health of the host (Wlazło et al., 2021). SB is widely used for centuries as a medicine in gastro – intestinal pathologies and diarrhea and also for improving cardiovascular and metabolic disease including obesity and diabetes (Suryakumar and Gupta, 2011; Wang et al. 2022). SB has also been previously found to promote the proliferation of beneficial bacteria, such as lactic acid and bifidobacteria in the gut and in that way to act as a prebiotic substrate (Attri et al., 2018; Wang et al., 2022). Also, as SB promotes the growth of probiotic organisms, it can be combined with them and provide a good synergetic supplement or feed (K. Wang et al., 2022). Varius *Lactobacillus* strains are used in therapeutic strategies, since they produce antimicrobial metabolites and reduce pathogenic bacteria in the gastrointestinal tract, and enhance desirable immune responses (Wlazło et al., 2021). In light of this fact, the present study's finding that SB supplementation promoted the enrichment of *Lactobacillus*, as it was most abundant in group A compared to the other diet groups, is of significant importance. Similar to previous reports, at the class level, *Clostridia* dominated the microbial communities in all the experimental groups (Bahl et al., 2020). In addition, *Shigella dysenteriae*, a gram – negative bacteria, cause of diarrhea in many animals and a zoonotic disease, was mainly detected from animals of group D, but without clinical signs of diarrhea. In contrast, the lowest abundance was in animals of group A, indicating that offspring as well as maternal SB supplementation may link to this beneficial effect. The results are in accordance with previous reports, where SB leaves have been shown to exert inhibition activity against these bacteria (Ren et al., 2020). Bacteria of the genus *Shigella* have also been found previously to dominate the intestinal track of both healthy (Zhao et al., 2017) and diarrheic mink; however, with greater abundance, in those with clinical symptoms of diarrhea (Birch et al., 2018). In our study, at the genus level the most abundant was *Romboutsia*. Although *Romboutsia* in general is considered as a gut inhabitant, its role in the gut of mink has not yet been explored. To further investigate the differences in microbial communities in mink, we then proceeded in DA analysis. Bacteria of the *Ruminococcaceae* family, which play a critical role in the maintenance of gut health (Gu et al., 2022), as also those of the *Lactobacillaceae* and *Leuconostocaceae* family, which belong to lactic acid bacteria (Collins et al., 1993), were detected in greater abundance in group A compared to D. A higher number of *Streptococaceae* bacteria was found in group A in comparison to D, but these bacteria are normal residents of gut microflora in canine species, as well as, in mink (Chalmers et al., 2015; Kruger et al., 2010). Moreover, bacteria belonging to the *Clostridiaceae* family were most abundant in control animals, descended from control dams (group D), compared to those which were descended from dams supplemented with SB (groups A and C). Similar results were also presented in a previous report in mice, where SB supplementation reduced the number of bacteria belonging to the same family (Yuan et al., 2018). Although limited, the direct and maternal effects of SB on microbial intestinal communities are of great interest, as they are depicted by the increase of beneficial bacteria and the decrease of those that are considered as harmful. Our results are in accordance with those of Panaite et al. (2022), who reported that feeding watermelon rids and SB on laying hens, promoted beneficial intestinal bacteria multiplication, while reducing harmful bacteria (Panaite et al., 2022). Similar results were also presented in previous studies in humans (Attri et al., 2018b; Chen et al., 2022), as well as in animals, such as mice (Guo et al., 2020; Lan, Sun, et al., 2022; Yuan et al., 2018), rats (Hao et al., 2019) and zebra fish (Lan et al., 2022).

To study the effect of SB on immune system and examine whether the alterations on intestinal microbial communities relate to alterations in colonic mucosa and systemic immunity networks, we then proceeded to immunohistochemical analysis of immune cells in colon, mesenteric lymph node (MLN) and spleen samples. We found evidence of reduction of T – lymphocytes in the colonic mucosa and mesenteric lymph nodes in animals that both they and the dams they derived from were supplemented with SB. We have also found some preliminary evidence

of a more systematic effect on immunity, since B – lymphocytes numbers were reduced in the spleen of animals that descended from dams fed with SB.

The inflammatory response of each organism typically exhibits a homeostatic balance between pro – inflammatory and anti – inflammatory signals, including signaling pathways, as also up – and down-regulation of specific cytokines. To examine further the effect of SB supplementation on immune system, we then proceeded to assess cytokines levels in blood serum and spleen samples. In accordance with previous studies, the bioactive compounds found in SB are implicated in the organism's anti – inflammation response, as they inhibit the production of the pro – inflammatory cytokines (Wang et al., 2022), while up – regulate some others (Lan, Wang, et al., 2022).

Sandwich ELISA was used to investigate the effect of SB supplementation in the mink diet on serum cytokine levels. Each group had a similar profile of the cytokines tested (IL – 4, IL – 8, and IFN – γ). Direct consumption of SB was found to stimulate the production of cytokines in mink to some extent. Specifically, group B animals showed statistically significantly higher levels of all three examined cytokines compared to group D animals, while concurrently, group A had higher IL – 4 levels than group D. On the other hand, when compared to group D, the levels of pro-inflammatory cytokines IL – 8 and IFN – γ decreased in groups that derived from dams receiving SB (groups A and C). The quantitative assessment of cytokine gene expression in spleen samples showed that the pro – Inflammatory IL1 – B in group A was significantly higher compared to control animals.

The anti-inflammatory cytokine, Tgf – β1 was lower in group B compared to the remaining groups, which is in accordance with a previous study, where Tgf – β1 levels in mice with alcoholic fatty liver disease were reduced after treatment with SB (Hong Zhao et al., 2022). However, Lan et al. (2022) reported opposite results in zebra fish after SB supplementation, where a decrease in the levels of IL1 – B and an up – regulation of Tgf – β1 were observed. Interestingly, the key pro – inflammatory cytokine IL – 6 in our study was higher in group A compared to D. Contrary to the results obtained by the ELISA analysis in blood serum, IFN – γ presented comparable values among the groups in spleen samples. Although elementary, and thus difficult to draw conclusions from, the results of the immunological analysis of the present research suggest that dietary SB consumption had an immunomodulatory effect on mink. This is clearly depicted by effects on specific immune cells and cytokines, not only in local gastrointestinal immunity networks, but also in the blood serum and spleen of mink. Based on the preliminary evidence of this study, further studies could be undertaken to adequately characterize the effects of dietary SB supplementation on the immune system of minks.

The findings of the present study fail to clearly support the hypothesis, that SB supplementation could enhance the reproductive traits of female mink, as no significant difference between the two groups was recorded. Nevertheless, aside the lack of statistical significance, the values of LS across the three repeated measurements were higher for animals supplemented with SB. Consequently, a higher number of kits within this group were finally weaned.

During the reproduction period, many fluctuations in the BW of female animals are typically observed. In our study, although BW of SB group was lower at the beginning of the experiment compared to control animals, after one month of SB supplementation (at the end of mating period – during embryo implementation), animals that received SB presented a higher BW gain compared to the Con group, balancing their initial BW difference. This fact is of particular significance, as it is assumed that mink supplemented with SB more efficiently utilized the provided feed during a period characterized by notably increased metabolic demands.

Beyond the reproductive traits of female breeders, the profitability of mink farms relies also on the quality and the size of produced skins, as the skin price is mainly configured by these two traits. Moreover, the expeditious preparation of the skin for pelting corresponds to the highest observed reduction in production costs for the farmer. For this purpose, we evaluated the effect of SB consumption on size and quality of skin, as also on hair follicle cycling of mink, hypothesizing that SB supplementation could accelerate the development of winter fur in mink. Considering that body length and body weight of animals in November are reliable indicators of pelt size (Thirstrup et al., 2017), we proceeded in measurements of BW during the whole growing period and measurement of body length before pelting. According to our records, body length of both male and female animals was similar for all the surveyed groups, regardless of the trial diet. Regarding BW, although many differences for female animals, were

observed, final BW was similar between the four groups, indicating that SB supplementation did not affect growth rate of female mink. Similar results are presented also by Solcan et al. (2013), who reported that supplementation with SB berries did not affect the final BW of chickens, as also by BenMahmoud et al. (2021), when 15% of wheat was replaced by sea – buckthorn fruits in broiler chickens' diet. An almost similar pattern was observed for male animals. However, the final BW for the animals that supplemented with SB and descended from control dams (group B) was lower compared to control animals (group C and D). Nevertheless, this reduction was not able to affect the final skin size and as a result, no difference was observed in the skins of male mink. Contrary to this, Ma et al. (2015) reported that broilers receiving flavones of SB fruits improved their BW, after 42 days of supplementation. Similarly, according to a previous study on sheep, the BW of animals was linearly increased with the dosage increase of SB (Hao et al., 2018), indicating that SB may exhibit dosage – dependent effect on BW.

The SB demonstrates considerable antioxidant activity and its oil is beneficial for skin health, as it promotes scavenging of ROS and cell proliferation, while at the same time, it improves skin elasticity, structure, and epidermal hydration (Zielińska & Nowak, 2017). Moreover, SB plays a critical role in wound healing, by epithelialization and fibroblastic deposition, as also wound contraction, after topical use (Gupta et al., 2006). In many previous studies, it is reported that SB is extensively used in cosmetics and cosmeceuticals, due to its anti – oxidative and anti – aging properties, as also due to its ability to promote growth, shiny and health hair (Pundir et al., 2021). However, the literature about the effect of systematic use of SB on skin is limited. According to previous reports, concurrent oral and topical SB oil treatment could expose anti – inflammatory and anti – psoriatic activity (Balkrishna et al., 2019). To study the effect of dietary SB supplementation on quality and hair growth of mink, we proceeded on subjective and objective assessment, including evaluation of hair re – growth, grading of fur quality on animals and skins, as also, measurement of skin thickness.

Both hair re – growth assessment and skin thickness by means of histomorphometry revealed no significant difference either for male or female animals of the experiment, indicating that supplementation with SB berries at 1% dosage on mink diet, did not influence the acceleration of hair follicle cycling. Similar to these, no difference was observed in the quality of both fur of animals and skins. However, skin price of female animals of group A was higher compared to the rest groups, which is maybe attributable to the overall improved appearance of the skins. Specifically, skins of female animals that both they and their female breeders were receiving SB were sold 3,5 – 4 € more than skins from females that either they or their breeders were fed with control diet.

The effect of 1% SB dietary inclusion on mink productivity and health was studied during an overall breeding season. Results indicate that SB supplementation at a dosage of 1% on mink improved to some extent some of the studied traits. Effects of SB supplementation in mink were observed in their immune status and intestinal microbiota. Nevertheless, reproductive traits, growth performance and skin traits were affected to a minimal extent.

CONCLUSIONS

Overall, Sea – buckthorn at a dosage of 1% constitutes a safe dietary supplement for mink, which positively affects to some extent their immune status, as also their bacterial intestinal microflora. Extra dosages of SB on mink diet should be included in investigations to develop an oriented plan for SB as a feed supplement for mink.

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CONFLICTS OF INTEREST

All authors have read and agreed to the published version of the manuscript. The authors declare that they have no conflict of interest to disclose.

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