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The Effect of Sodium Humate Feed Additives in Diets for Holstein Breed Heifers

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Abstract

The research aim is to examine the possibilities of including the sodium humate (NaHum) additive derived from freshwater sapropel in feed to identify its effects on growth performance, promote haematopoiesis, and modulate the microbiota of the intestinal tract. The research was done under production conditions, complying with Latvian and European Union legislation on the keeping, feeding, and welfare of farm animals. The research had three replications, and for each of them, two groups of Holstein breeding heifers were established: control (3xn=7) and research (3xn=7). The duration of each replication was 9 days in the adaptation period and 105 days in the research period. The heifers of the research group received the NaHum solution additive with feed from the 1st to 35th day (stage 1) at an intake rate of 0.4 mL/kg of live weight, from the 36th to 70th day (stage 2) at an intake rate of 0.5 mL/kg of live weight, and from the 71st to 105th day (stage 3) at an intake rate of 0.6 mL/kg of live weight. The breeding heifers of the research group, receiving NaHum at an intake rate of 0.6 mL/kg of live weight, achieved a significantly higher live weight gain at stage 3 and an overall numerically higher live weight gain (by 4.8 kg) than the control group during the research period. Consequently, a significantly higher relative growth ratio (0.334) was found in the research group at stage 3, which was 0.028 higher than that in the control group. The *Lactobacillus* spp. count in faecal samples was steady at the end of the research; a significant difference was found between groups, with the average ranging between 6.95 (control group) and 8.49 log CFU/g (research (NaHum) group). The novelty of the research is that it was scientifically proven that feeding the NaHum additive derived from freshwater lake sapropel to the breeding Holstein heifers up to 5 months of age increased their feed intake and live weight gain, as well as activity and health.

Keywords:
Sodium Humate;
Feed Additives;
Growth Performance;
Blood Parameters;
Microbiota;
Intestinal Tract;
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1- Introduction

By 2050, the world is projected to have a significant shortage of food, as there will soon be 10 billion people on Earth—about 3 billion more than in 2010 [1]. Therefore, innovative strategies are needed to contribute to the sustainability of livestock production and consumption systems [2, 3]. Meat and milk produced from ruminants represent important sources of protein in the human diet worldwide [4]. Livestock production supports local trade and the regional economy, and in rural areas, livestock production not only generates income for farm owners, but is also a source of food and labour [5–7]. In many parts of the world, scientists and producers are seeking new strategies and technological innovations to improve the nutrition, genetics, and health of farm animals to increase productivity in livestock farming and allow for a more efficient use of resources [8, 9]. Productivity and income are essential for sustainable agricultural

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growth [10]. Concerns about the sustainability of agricultural systems relate to the need to develop new technologies and practices that would not negatively impact the environment, would be effective and accessible to farmers, and would increase the efficiency of food production [11]. Increasing agricultural productivity is the only solution to supplying food to the growing world population [12, 13]. Increasing feed absorption efficiency is crucially important in the production of meat and milk. Feed composition and quality are the main factors that influence the productivity of farm animals and feed absorption efficiency [14, 15]. Increasing livestock productivity (i.e., increases in live weight or milk yield per farm animal) and fertility (i.e., less culling and lower replacement rates) can reduce GHG emissions per kg of livestock products produced [16]. In addition, it is necessary to seek solutions to enhance the health of livestock, as this could increase their productivity and longevity by using current resources more efficiently [17].

Sodium humate inclusion in the diet of breeding heifers effectively decreases diarrhea incidence and improves growth performance in pre-weaned Holstein calves by enhancing their antioxidant and immune status and modulating intestinal microflora [18]. Sodium humate is a macromolecular substance comprising humic acid, which is rich in phenolic hydroxyl and carboxyl groups and has antidiarrheal, antioxidant, and anti-inflammatory properties. Moreover, humic acid inhibits Escherichia coli and other pathogenic micro-organisms [19]. This is in line with the European Green Deal and its central "Farm to Fork" strategy, which multifacetedly addresses challenges for sustainable food systems [20]. In addition, the European Commission has emphasized that in the European Union (EU), raw materials should be produced from local resources as much as possible [7]. In the period of 2017–2022, scientists from Latvia University of Life Sciences and Technologies (LBTU) sought opportunities to create new, innovative feed additives from sapropel deposited in a freshwater lake, which would increase productivity in the livestock industry while having beneficial effects on the health of farm animals. Sapropel is composed of the remains of animals and plants living in freshwater bodies; it consists of organic substances and an admixture of sand, clay, and carbonates that make up 5-85% of the mass. In some countries, significant research has been conducted on the extraction and chemical composition of sapropel, finding that sapropel represents more than 30 macro and microelements [21–33].

In Latvia, lakes also have significant reserves of sapropel sediments [34], which are estimated at approximately 800-900 million m^3, and these resources as raw materials could last for several centuries [35]. Similar research studies have also been done in several European countries, with emphasis placed on the content of humic substances in sapropel. Research on sapropel is especially important for the Baltic States and Northern Europe because, in this region, sapropel is widely distributed and available in freshwater pools [26]. Research on sapropel has been conducted in Belarus [27], Sweden [28, 29], Romania [30], Croatia [31], Germany [32], Norway [33], and other countries. Several research studies on sapropel and its potential uses have also been done in the Baltic States [36–39]. As most of the scientists have focused their research on the chemical composition of sapropel, there are relatively few research studies on the uses of sapropel and its biologically active principles in the economy. In Latvia, several research studies have been conducted on the use of sapropel in construction [35, 40, 41], on producing sapropel extract and its use in the manufacture of pharmaceutical preparations [42], and on the use of sapropel in soil fertilization and improvement [26, 43]. However, only two research studies, published in 2001, have been conducted on the use of sapropel in livestock farming, and they concern the consumption of sapropel additives by poultry and pigs [44, 45]. Therefore, the findings of this research on the inclusion of sodium humate feed additives derived from lake sapropel in diets for breeding Holstein heifers are unique, as they can increase milk and beef cattle production based on local resources, thereby strengthening the health of farm animals and contributing to the sustainable development of respective agricultural industries.

According to the Food and Agriculture Organization of the United Nations, the global consumption of dairy products is expected to increase by 63% by 2050 compared with 2005/2007 [46]. According to a projection, a total of 1085 thousand tonnes of milk will be produced in Latvia in 2030, a number that is expected to rise to 1130 thousand in 2050 [47]; quality heifer rearing is crucial to increasing milk production. This research put forward the following hypothesis: the addition of a NaHum additive derived from fresh-water sapropel can result in various positive effects on breeding heifers, such as a higher growth rate, better health, and a regulated microbiota in their intestinal tracts. Therefore, the present research aims to examine the possibilities of including the NaHum additive derived from freshwater sapropel in feed to identify the effects on growth performance, promote haematopoiesis, and modulate the microbiota of the intestinal tract for breeding Holstein heifers.

The novelty of the research is that it was scientifically proven that feeding the NaHum additive derived from freshwater lake sapropel to the breeding Holstein heifers up to 5 months of age increased their feed intake and live weight gain, as well as activity and health.

2- Research Methodology

A step-by-step methodology for the dietary research is presented in Figure 1.

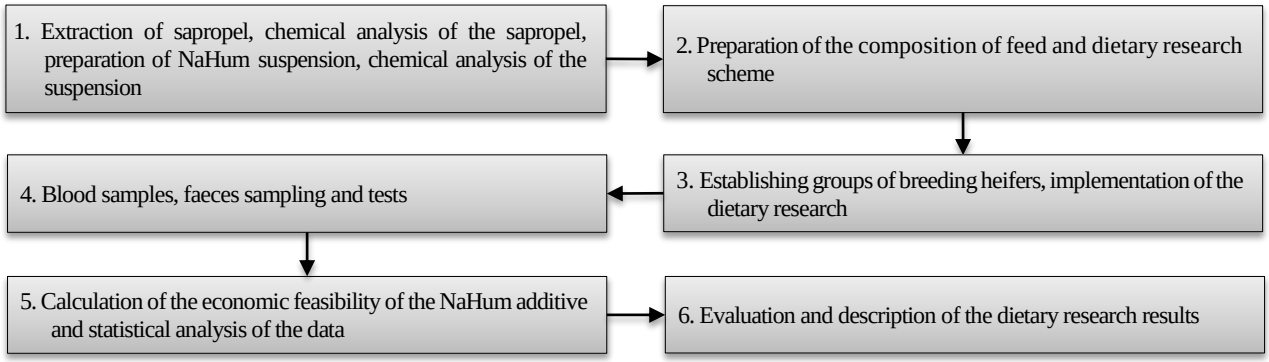


Figure 1. Research scheme and its main elements (steps)

Detailed methodology of the dietary research is explained in Sections 2-1, 2-2, 2-3, 2-4, 2-5 and 2-6. Research results are summarized in Section 3.

2-1- Characteristics and Extraction Methodology of Sapropel and NaHum

The research used organogenic silicate sapropel extracted from Lake Bizas, located in southeastern Latvia, Kraslava Municipality, Andrupene Parish. The surface area of the lake is 142.29 ha, while the surface area together with its five islands is approximately 144 ha; the sapropel reserves that could be extracted at a shallow depth (on average 1.5 m) amounted to 6.5 mln. M^3, of which 4567.8 thou. M^3 or 1 190.72 thou. T (at moisture content W = 60%) of N category sapropel reserves were approved for extraction. In the lake, the sapropel layer is 0.4–2.7 m thick. The lake is located on the western edge of the Latgale highland, in the Feimanu moraine hummock region, and could be classified as a flow-through lake. However, the artificial lowering of the water level leads to intense eutrophication processes in it because the lake is overgrown; therefore, industrial sapropel extraction would also benefit the lake ecologically. According to the main type, the lake could be classified as eutrophic (rich in nutrients). The areas adjacent to the lake are partly swampy, interspersed with agricultural land and forests [48].

NaHum is a liquid of viscous consistency and resembles molasses: dark brown in colour with a specific smell [49] (Figure 2). It was extracted from organogenic silicate sapropel according to the protocol developed by the Humic Substance Society [50], applying this technique for the extraction of humates from sapropel. The humic substances were already present in the sapropel, and a chemical method was used to extract them. In the process, sodium hydroxide (NaOH) was used to dissolve and extract the humic substances from the sapropel. The weight and absolute moisture of dry sapropel were calculated for the sapropel samples. Based on the data, 1000 ml of 0.5 M NaOH solution or 2% NaOH aqueous solution was prepared per 100 g of absolutely dry sapropel. The prepared suspension was stirred on a magnetic stirrer at 850 rpm for 4 h at 85 °C. As a result, the suspension consisted of a NaHum solution and a water-insoluble mineral component, which consisted of clay minerals and primary minerals such as quartz, feldspar, etc. To separate the mineral component, centrifugation was carried out 1-3 times (as needed), spinning the sample for 15 minutes at 3200 rpm, thereby obtaining the NaHum supernatant, which was poured into a separate container. The obtained NaHum solution was stored in a closed container in the dark at 4 °C.

Figure 2. (a) A map of Latvia with a sapropel extraction site in 1 – Lake Bizas (56°10'10.5"N 27°22'06.5"E) and the location of the 2 – farm (56°79'37.5"N 24°68'48.9"E) where the dietary research was conducted, (b) NaHum derived from sapropel

There are many potential applications for humates that can be extracted from the widely distributed sapropel resources in Latvia. The extraction of humates represents a good opportunity to save time and resources since it does not require complete dehydration of sapropel. Moreover, this method can simplify the complex process of sapropel dehydration, which has hindered the efficient use of these resources in the national economy.

2-2- Starter Feed, NaHum Samples and Tests

The chemical composition of NaHum and starter feed was examined at the LBTU Research Laboratory of Biotechnology (accreditation No. LATAK-T-168) using generally accepted testing techniques. In this research, we use the unit g/kg of dry matter to express the nutrient content of the samples being analyzed. This unit of measurement was chosen because of the varying levels of moisture content between NaHum and feed samples and allowed for more accurate comparisons of nutrients between them. The sapropel samples were obtained from geotubes after six months of dehydration. The collected samples were tested for pathogens and various nutrients (as shown in Table 1). The NaHum was extracted from the sapropel using the methodology described above.

Table 1. Starter feed given to the heifers and NaHum testing methods

Nutrients	Method applied
Dry matter (DM), g	LVS EN ISO 6498:2012;7.5
Crude protein, g/kg DM	LVS EN ISO 5983-2:2009
Crude fibre, g/kg DM	ISO 5498:1981
Exchangeable energy (ME), MJ/kg DM	* Calculation method
Net energy for gain (NEG), MJ/kg DM	* Calculation method
Crude fat, g/kg DM	ISO 6492:1999
Crude ash, g/kg DM	ISO 5984:2002/Cor 1:2005
Calcium (Ca), g/kg DM	LVS EN ISO 6869:2002
Phosphorus (P), g/kg DM	ISO 6491:1998
Potassium (K), g/kg DM	*LVS EN ISO 6869:2002
Magnesium (Mg), g/kg DM	LVS EN ISO 6869:2002
Sodium (Na), g/kg DM	LVS EN ISO 6869:2002
Zinc (Zn), g/ kg DM	*LVS EN ISO 6869:2002
Copper (Cu), g/kg DM	*LVS EN ISO 6869:2002
Manganese (Mn), g/kg DM	*LVS EN ISO 6869:2002
Iron (Fe), g/kg DM	*LVS EN ISO 6869:2002
Starch, g/kg DM	LVS EN ISO 10520:2001
Dry matter digestibility (TDN/DDM**), %	* Calculation method
Organic matter digestibility (OMD), %	* Cellulase method
Dry matter intake (DMI), %	* Calculation method
pH	GOST 26180-84, met.3

* Unaccredited method; **TDN -Total Digestible Nutrients, DDM- Digestible Dry Matter

The composition of starter feed was determined at the start of the dietary experiment to compare it with that specified in the manufacturer's recipe. Starter feed tests were not performed for the replications. NaHum tests were performed before each replication. All chemical test results were reported on a dry matter basis.

2-3- Principles of Establishing Groups of Breeding Heifers, A Dietary Scheme for The Groups

The present research complied with the requirements of the Law of the Republic of Latvia (RoL) (2007) "On the European Convention for the Protection of Animals Kept for Farming Purposes and the Protocol" [51]. The research was carried out in cooperation with an agricultural enterprise, Ogres piens Ltd, in its livestock shelter in Latvia, Ogre Municipality, Ogresgals Parish (address: Ogre, LV-5041 (Figure 1)), Vidzeme region, where the NaHum additive derived from freshwater sapropel was fed to breeding Holstein heifers under production conditions. Observations and data were recorded and blood samples were collected during routine work on the farm, and, according to EU Directive 2010/63/EU, Article 1, Paragraph 5, Point a [52], approval from the ethics commission for non-experimental agricultural practices was not required. The research location was a dairy farm with 700 dairy cows. The average milk yield per cow in the monitoring year of 2021 was 12,389 kg, the average fat content of milk was 3.62%, the protein content was 3.35%, and the count of somatic cells was 106,000 cells/mL [53].

For research purposes, 2 groups (control and research) of the Holstein black and white breed heifers were established. Three replications of the dietary research were conducted to ensure the reliability of the research results. The first replication was carried out from 6 October 2021 to 19 January 2022 (control group n=7, research group, n=7), the second replication from 10 November 2021 to 23 February 2022 (control group n=7, research group, n=7), and the third replication from 09 February 2022 to 25 May 2022 (control group n=7, research group, n=7). In all the replications, a total of 21 breeding heifers were used in the research group and 21 in the control group. The research results represent data from all the three replications. The duration of each replication was 9 days for the adaptation period and 105 days for the research period, which was divided into 3 stages based on daily NaHum dose: stage 1 from the 1st to 35th day, stage 2 from the 36th to 70th day, and stage 3 from the 71st to 105th day (Table 2).

Table 2. Intake of NaHum by research group and by stage of the dietary research

Research stages	Duration of stage (days)	Intake of NaHum per kg of live weight (mL)
Adaptation period	9	0.3
Stage 1 (day 1st to 35th)	35	0.4
Stage 2 (day 36th to 70th)	35	0.5
Stage 3 (day 71st to 105th)	35	0.6

The breeding heifer groups were established, and the necessary data were recorded by the farm's specialists. The dietary research used heifers that were born with a live weight of 35 kg or more and were intended for reproduction in the cow herd. When establishing the research groups, the heifers were selected so that their average age was 39-40 days for all the three replications at the beginning of the research. At this time, the heifers of the control group were one day older than the heifers of the research group, and this difference in age between the groups remained at all stages of the research. During the research, the breeding heifers were kept in groups in outdoor sheds and fed in accordance with Cabinet regulation of the RoL (2003) No. 491 Calf Welfare Requirements [54], providing each group with a separate box with an area of 17.5 m^2, i.e., 2.45 m^2 per heifer, which met heifer welfare requirements. Straw bedding was available in the boxes, starter feed and hay were fed from troughs without limit, and milk was fed individually from buckets. Starter feed (ab libitum) and hay (ab libitum) were fed to the heifers of the control and research groups throughout the research. Milk was rationed from 9 L at the beginning of the research (1st day of stage 1) to 2 L on the 56th day of the research (21st day of stage 2).

The heifers of the research group additionally received the NaHum additive. When feeding the heifers with milk, NaHum was added to the milk; after milk feeding was finished, NaHum was mixed manually into the starter feed, dividing the feed ration into two parts and giving one part in the morning and the second one in the evening. After the heifers had consumed starter feed supplemented with NaHum, the starter feed was replenished in the trough to ensure unlimited intake. According to the research protocol, the farm specialists recorded the amounts of feed and the additive consumed every day.

To gradually accustom the heifers to the new dietary and housing conditions, an adaptation period (9 days) was applied before starting the dietary research. During this period, the heifers were fed a small amount of NaHum at an intake rate of 0.3 mL of NaHum per kg of live weight. At the beginning of the experiment—at stage 1 of each replication—the heifers were fed 0.4 mL of NaHum per kg of live weight, gradually increasing it to 0.6 mL at stage 3 (Table 2), which was in line with the methodology for dietary experiments conducted by scientists from other countries [55]. During the experiment, the rate of intake of NaHum was adjusted every 5 days according to the daily live weight gain of the heifers in the previous period and the expected live weight.

The breeding heifers were weighed every 35 days (the duration of one research stage). Electronic scales with an accuracy of 0.01 kg were used for the weighing. The research used the data obtained for live weight gains during both the research stages and the entire research period, the average daily live weight gain, and the amounts of feed consumed to make group comparisons. For the calculation of feed consumption for one heifer during the research, the obtained data on the total amount of feed consumed by a group of heifers and the feed days during which this feed was consumed were used.

The live weight gains were calculated according to the following equation [56]:

$$WG = BWe - BWi \quad (1)$$

where WG is weight gain, BWe is the live weight at the end of the research period, and BWi is the initial live weight.

An average daily live weight gain (ADWG) per animal was calculated according to the following equation [56]:

$$ADWG = \frac{BWe - BWi}{T} \quad (2)$$

where T is the research period in days.

2-4- Blood Samples and Tests

At the end of the research (on the 105th day), a farm veterinary medicine specialist took two blood samples from each breeding heifer (each on average 10 mL) as part of the animal health monitoring according to the plan of the farm. The blood samples were taken from the jugular vein about 3 h after morning feeding and, after being stored in a +4 °C environment, delivered to the laboratory within 4 h. At the laboratory of the LBTU Veterinary Clinic, serum was derived from one blood sample by centrifuging it at 4000 rpm for 20 min. The serum was stored in 1.5 mL Eppendorf tubes at -20 °C until further investigation. The following characteristics were identified for the serum by employing the absorption photometry method according to the recommendations of the reagent Accent - 200 manufacturer (PZ CORMAY S.A., PL): total cholesterol (CHOL, kat.nr. 7-204), total protein (TP, kat.nr. 7-236), albumin (ALB, kat.nr. 7-238), globulin (GLOB), alkaline phosphatase (ALP, kat.nr. 7-212), phosphorus (P, kat.nr. 7-243), calcium (Ca, kat.nr. 7-247), magnum (Mg, kat.nr. 7-229), and triglycerides (TRIG, kat.nr. 7-273) by means of a clinical chemistry analyzer (Mindray BS-380, Shenzhen Mindray Bio-Medical Electronics Co. Ltd, PRC). Glucose (GLU) was measured with an ACCU-CHECK Instant glucometer (Roche, CH). Haematological parameters were identified for the blood samples anticoagulated with Ethylenediamin tetra-acetic acid (EDTA). The following characteristics were identified using the photometry method and a veterinary haematological analyzer (Exigo Eos, Boule Medical AB, Sweden): white blood cells (WBCs), red blood cells (RBCs), monocytes (MOs), lymphocytes (LYMs), haematocrit (HCT), and haemoglobin (Hb).

2-5- Faeces, Aapropel Aampling and Tests

On the last day of the research before the morning feeding of heifers, faecal samples (approximately 50 g per animal) were directly collected by stimulating the rectum. The samples were placed in sterile bags, stored in a +4 °C environment, and delivered to the laboratory within 4 h. Tests on faecal microbiota were performed within 2 h after the samples were delivered to the laboratory. The faecal contents were used for identifying the counts of *Enterobacteriaceae*, *Lactobacillus* spp., and *Clostridium perfringens*. Initial and serial dilutions of the samples were made in peptone saline (Maximum Recovery Diluent, Biolife, IT) according to ISO 6887-1:1999. For the isolation and enumeration of the *Enterobacteriaceae* bacteria, VRBG (Violet Red Bile Glucose agar, Biolife) was used and tested according to ISO 21528-2:2007, while for enumeration of coliforms MacConkey agar (Oxoid) was used.

PCA (Plate count agar, Oxoid) was used for determining total viable and total anaerobic bacteria counts according to LVS EN ISO 4833-1. For the isolation of Gram-positive microorganisms, a selected medium (CNA agar, Becton Dickinson (BD)) was used according to the manufacturer's instructions. *Salmonella* spp. in the sapropel samples was tested according to LVS EN ISO 6579-1. The isolation and enumeration of *Lactobacillus* spp. were performed using MRS agar with Tween 80 (Biolife) and according to the medium manufacturer's instructions. The incubation was realized at 36 °C ±1 °C, for 72 h ±2 h. The research confirmed the most typical colonies by performing a Gram staining and catalase test. TSC (Tryptose sulfite cycloserine agar, Biolife) was used for the isolation of *Clostridium perfringens* bacteria. The prepared faecal dilution plates were incubated under anaerobic conditions at 36° C ±1 °C, for 24 h ±1 h in a BD Gas-Pak EZ container system with BD BBL CO_2 generators and indicators. The isolated strains suggestive of *C. perfringens* were further identified by Gram staining and with Vitek MS, MALDI-TOF (bioMerieux SA, FR). The obtained results were calculated and expressed as log10 colony-forming units per gram of faecal contents.

The microbiological test conducted on the partially dehydrated sapropel samples showed the absence of *Enterobacteriaceae* bacteria and *Salmonella* spp. This indicated that the raw material (sapropel) used for NaHum extraction did not contain these specific pathogens. Therefore, the obtained sapropel could be considered suitable to produce NaHum as a feed additive for livestock farming. Furthermore, the NaHum extraction process involved heating the sapropel at 85 °C for 4 h, which might have further reduced the likelihood of any potential pathogens surviving in the resulting product. To minimize the risk of cross-contamination, proper storage of NaHum was achieved in a closed container to prevent moisture from entering and causing clumping or caking. The NaHum samples were kept in the dark at 4 °C, and the NaHum was stored separately from other feed ingredients to avoid any potential interactions that could impact its effectiveness.

2-6- Parameters and Methods Used for Identifying the Economic Feasibility of the NaHum Additive

The economic feasibility of the NaHum additive was identified using indicators such as live weight gain (WG), quantity of feed consumed (QF), and feed cost (FC), calculating feed cost per average daily weight gain (ADWG) according to the following equation:

$$FC_{ADWG} = \frac{\sum_{1+n}(QF_1 \times P_1) + \cdots + (QF_i + P_i)}{ADWG} \quad (3)$$

and feed cost per total live weight gain (WG) during the research period according to the following equation:

$$FC_{WG} = \frac{\sum_{1+n}(QF_1 \times P_1) + \cdots + (QF_i + P_i)}{WG} \quad (4)$$

where QF is the quantity of feed and P is the feed price.

Since the growth of breeding heifers is an important indicator of the reproduction of a high-quality and high-yielding herd, the relative growth ratio (RGR), kg of BW per day, or the exponential growth rate was identified according to Lima et al. (2017) [57]:

$$RGR = \left[\frac{(\log BWef - \log BW_{i./f})}{T}\right] \times 100 \quad (5)$$

The authors used RGR to compare relative growth ratio of breeding heifers between research groups, as well as to evaluate changes in the relative growth ratio within each group at different stages of the research.

The farm's data on feed and milk costs in the period from September 2021 (beginning of the dietary research) to May 2022 (end of the research) were used in the calculations. The farm's average selling price of fresh milk was used to calculate the cost of milk fed to the heifers. The price of starter feed was calculated based on the average market price during the research period. However, NaHum cost calculations considered the market price of analogue feed additives (GreenOK NaHumates for animals) in the period 2021/2022 [58].

2-7- Statistical Analysis of the Data

All the obtained research data such as feed intake, growth performance, blood and faecal parameters were analyzed by one-way ANOVA using the R Studio software (version 4.1.2.). Statistically significant differences between the groups were analyzed using Tukey's HSD test. Statistical significance was set at $P < 0.05$. To indicate the spread of variance around the arithmetic mean, the data are presented as means ± SD (standard deviation).

3- Results

This section describes the results of the dietary research: 1) the composition of the basic feed as well as the NaHum; 2) the growth and live weights of the breeding heifers at various ages, changes in live weight gain during the day; 3) the consumption of feed and additives at various stages of the research and the economic feasibility of the NaHum additive; and 4) the results of laboratory tests on the blood and faeces of the breeding heifers to identify their general health status.

3-1- The Composition of Basic Feed as Well as NaHum (Key Components)

To identify the potential suitability of sapropel for the needs of livestock farming, a microbiological test on the samples of partially dehydrated sapropel (abs. moisture 83%) was initially done in the laboratory. The microbiological characteristics of sapropel (log CFU/g, average ±standard deviation (SD)) were as follows: 1) total aerobic bacteria count 6.5 ±0.25; 2) total anaerobic bacteria count 3.2 ±0.12; 3) Gram-positive bacteria count 4.23 ±0.014. Among the above characteristics, the highest one was the total aerobic bacteria count: on average 3.7x106 ±1.9x106. The average Gram-positive bacteria count was 1.7x104 ±5.5x102, while the total anaerobic bacteria count was 1.65x103 ±4.5x102. The research did not detect the presence of *Enterobacteriaceae* bacteria and *Salmonella* spp. in the sapropel samples taken. The obtained results allowed the NaHum derived from the sapropel extracted to be used as a feed additive. NaHum was produced under laboratory conditions by adding NaOH to the sapropel.

Table 3. Chemical composition of NaHum and starter feed used in the dietary research

Nutrients	NaHum	Starter feed
Dry matter (DM), g	44.8	885.3
Crude protein, g/kg DM	104.3	224.6
Crude fibre, g/kg DM	3.6	33.1
Exchangeable energy (ME), MJ/kg DM	14.3	14.2
Net energy for gain (NEG), MJ/kg DM	5.8	6.0
Crude fat, g/kg DM	0.9	59.5
Crude ash, g/kg DM	759.8	63.5
Calcium (Ca), g/kg DM	4.2	6.2
Phosphorus (P), g/kg DM	0.8	4.6
Potassium K, g/kg DM	2.6	-
Magnesium (Mg), g/kg DM	1.7	-
Sodium (Na), g/kg DM	4.2	-
Zinc (Zn), g/kg DM	0.09	-
Cooper (Cu), g/kg DM	0.01	-
Manganese (Mn), g/kg DM	0.06	-
Iron (Fe), g/kg DM	4.81	-
Starch, g/kg DM	-	419.5
Dry matter digestibility (TDN/DDM*), %	82.3	83.8
Organic matter digestibility (OMD), %	87.0	91.6
Dry matter intake (DMI), %	113.2	8.7
pH	13.0	-

*TDN- Total Digestible Nutrients, DDM- Digestible Dry Matter.

It was found that NaHum contained an average of 4.48% dry matter; a kg of dry matter contained 104.3 g of crude protein, 3.6 g of crude fibre, and 0.9 g of crude fat; 14.3 MJ of metabolic energy and 5.8 MJ of energy for live weight gain; and a very high content of crude ash at 759.8 g, including 4.2 g Ca, 4.2 g Na, and 4.81 g Fe (Table 3). The dry matter digestibility of NaHum was 87.0%, and the absorption capacity of dry matter was 113.2%. The pH level was 13.0, i.e., alkaline. An analysis of the chemical composition of the starter feed revealed that it contained 88.5% dry matter, while a kg of dry matter contained 224.6 g of crude protein, 33.1 g of crude fibre, 59.5 g of crude fat, and 419.5 g of starch; 14.2 MJ of metabolic energy and 6.0 MJ of energy for live weight gain; and 63.5 g of crude ash, including Ca 6.2 g and P 4.6 g (Table 3).

3-2-Growth of the Breeding Heifers by Stage of The Research and Throughout the Research Period

The breeding heifers of the control group were on average 40±8.1 days old at the beginning of the research (Table 4), while the heifers of the research group were 39 ±7.1 days old. In view of the duration of each stage, the age difference of one day between the groups of breeding heifers was the same throughout the research period. The breeding heifers that were fed the NaHum additive had a numerically higher live weight than the control group at all the stages of the research, yet the difference was not significant (Table 4).

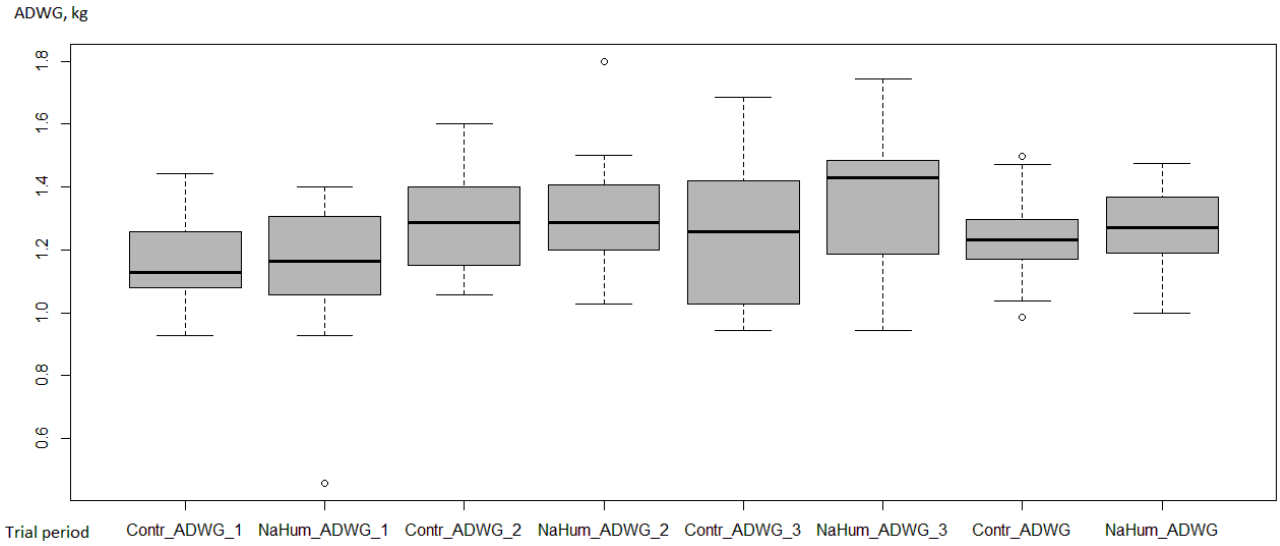
Table 4. Ages and live weights of the breeding heifers at various stages of the dietary research

Research stage	Age, days				Live weight, kg			
	Group		P-value	Difference to control	Group		P-value	Difference to control
	Control (n=21)	Research, NaHum (n=21)			Control (n=21)	Research, NaHum (n=21)		
Birth	0	0		0.0	39.0 ±4.17	39.7 ±2.90	0.274	0.7
Begin of stage 1	40 ±8.1	39 ±7.1	0.463	-1.0	69.9 ±10.18	71.0 ±10.02	0.360	1.1
Begin of stage 2	75 ±8.1	74 ±7.1	0.463	-1.0	110.6 ±12.48	111.0 ±15.18	0.458	0.5
Begin of stage 3	110 ±8.1	109 ±7.1	0.463	-1.0	155.9 ±15.63	156.4 ±17.54	0.464	0.5
At the end of research	145 ±8.1	144 ±7.1	0.463	-1.0	199.5 ±21.01	204.4 ±19.99	0.222	4.8

* Data are presented as means ± SD—standard deviation.

The daily live weight gains of the breeding heifers in all the groups are shown in Figure 3. The control groups had a variable ADWG, from 1.163 kg per day at stage 1 to 1.296 kg at stage 2, followed by a slight decrease at stage 3 to, on average, 1.246 kg (Figure 3). The breeding heifers of the second research group had live weight gains at all the stages, which ranged from 1.144 kg at stage 1 to 1.371 kg at stage 3. During the entire research period, the live weight of the heifers in this group increased on average by 1.270 kg per day, which was 35 g more than that of the heifers in the control group. An increase in intake of NaHum to 5 g for the research group resulted in the highest daily live weight gain (Figure 3).

During the 35 days of stage 1, the heifers of the control group had a 0.65 kg higher live weight gain than the research group (Table 5). At stage 2, the live weight gain was the same in both groups, on average 45.35 kg, while at stage 3, the breeding heifers of the research group achieved a significantly higher live weight gain, on average 47.98±1.75 kg, which was 4.37 kg more ($P<0.05$). During the entire research period, the numerically highest live weight gain was achieved by the research group.



*ADWG_1—average daily weight gain during stage 1; ADWG_2—average daily weight gain during stage 2; ADWG_3—average daily weight gain during stage 3; ADWG—average daily weight gain during the entire research period.

Figure 3. Daily live weight gains of the breeding heifers during the dietary research, kg/day

Table 5. Average live weight gains of the breeding heifers during the dietary research

Group	Live weight gain, kg						
	Stage 1 (35 days)	Stage 2 (35 days)	Stage 3 (35 days)	Difference; P-value			Total (105 days)
				Stage1/Stage 2	Stage 1/Stage 3	Stage 2/Stage 3	
Control (n=21)	40.70^{Aa} ±4.91	45.35^{Bb} ±6.09	43.61^{ABab} ±8.06	4.65; P=0.003	2.91; P=0.073	-1.74; P=0.201	129.66±13.87
Research (NaHum) (n=21)	40.05^{Aa} ±7.55	45.35^{Bb} ±6.11	47.98^{BCb} ±7.82	5.30; P=0.009	7.93; P=0.001	2.63; P=0.122	133.38±13.56
Difference	-0.65	0.00	4.37	-	-	-	3.71
P-value	0.328	0.499	0.039	-	-	-	0.226

A, B, C—significant difference between stages within group; a, b, c—difference in live weight gain is significant between the groups (P≤0.05); *Data are presented as means ± SD—standard deviation.

It was concluded that increasing the intake of the NaHum additive to 0.6 mL per kg of live weight contributed to the growth rate of the breeding heifers, yet a lower rate of intake was not effective. According to the farm's specialists, in the group that was fed the NaHum additive, the appearance of the breeding heifers indicated their good health, with bright eyes, smooth and shiny coats, and active behaviour indicating their welfare.

3-3- Quantities of Feed and The Feed Additive Consumed During the Dietary Research and The Economic Feasibility of the Nahum Additive

At stage 1, the breeding heifers of both groups consumed equal quantities of milk, on average 7.271 and 7.274 L, which could be explained by the rationed consumption of this feed (Table 6).

Table 6. Feed consumption per day by stage of the dietary research

Research stage	Control group (n=21)		Research, NaHum group (n=21)		P -value	
	Starter feed	Milk	Starter feed	Milk	Starter feed	Milk
Stage 1	0.874^{a} ±0.405	7.271^{a} ±1.799	0.755^{b} ±0.284	7.274^{a} ±1.784	0.016	0.497
Stage 2	4.079^{a} ±1.210	3.028^{a} ±0.746	4.145^{a} ±1.250	2.830^{a} ±0.760	0.433	0.201
Stage 3	5.221^{a} ±0.291	not consumed	5.665^{b} ±0.651	not consumed	0.000	not consumed

* Data are presented as means ± SD—standard deviation; a, b—difference in feed consumption is significant between the groups, P≤0.05

The starter feed was not rationed, and the breeding heifers of the control group consumed significantly more of it, on average 0.874 kg, 0.119 kg more than the breeding heifers of the research group (P≤0.05) (Table 6). This could explain the higher live weight gain (0.65 kg) of the control group at this stage. At stage 2, the breeding heifers of the research group consumed more starter feed (0.066 kg) than those of the control group, yet they consumed less milk (0.198 L). This indicated that the rumens of the breeding heifers of the control group were already better developed, and they could get the necessary nutrients from the starter feed.

At stage 3, the starter feed fed to the breeding heifers continued to be unlimited. The results indicated that the breeding heifers of the control group consumed on average 5.221 kg per day during the 35-day period, while those of the research group consumed on average 5.665 kg, which was 0.444 kg more (P≤0.05). The quantities of feed consumed during the dietary research are shown in Table 7. Hay was fed in the same quantity to all the groups, at 25.9 kg per heifer during the 105-day research period.

Table 7. Starter feed and milk consumption per breeding heifer by group during the dietary research

Indicator	Group		Difference against control
	Control (n=21)	Research, NaHum (n=21)	
Starter feed, kg	356.11	367.01	10.90
Milk, L	318.0	314.0	-4.0

During the dietary research, breeding heifers of the research group consumed on average 10.9 kg of starter feed more and 4 L less milk than a heifer of the control group. Heifers of the research group were additionally given an average of 5887.75 mL of NaHum in 105 days; at stage 1, each heifer ingested 25.8 mL of NaHum every day (Table 8), which was increased at each subsequent stage, and at the final stage, it amounted to an average 89.6 mL per day, which provided an additional intake of 4 g of dry matter.

Table 8. Average intake of the NaHum additive by stage of the dietary research

Research stage	Amount of NaHum during stage, mL	NaHum per heifer per day, mL	NaHum dry matter per heifer per day, kg
Stage 1	516.0 ±120.9	25.8 ±4.05	0.001 ±0.000
Stage 2	1056.4 ±266.9	52.8 ±13.35	0.002 ±0.001
Stage 3	1792.0 ±186.5	89.6 ±9.32	0.004 ±0.000

During the three replications of the research, in addition to basic feed, the breeding heifers of the research group were fed 5.56 kg of NaHum dry matter, on average 2.5 g per day. This provided the breeding heifers with additional proteins and minerals of natural origin, such as Mg, Na, Fe, etc., which are necessary for the functioning of the calf body, thereby having a positive effect on their health and growth. Therefore, research on NaHum feed additives derived from lake sapropel should be continued, additionally extracting the dry fraction, which could provide a simpler method of dosing NaHum in the livestock feeding process.

The absolute values of feed cost (Table 9) per breeding heifer were higher for the research group because of the higher starter feed consumption and NaHum cost for the control group. The total feed cost per breeding heifer for the research group during the entire research period was equal to EUR 28.59 or 7.88% higher than for the control group. However, a calculation of feed cost per average daily weight gain (ADWG) and average weight gain (WG) during the research period revealed that the cost for the research group (NaHum) exceeded that for the control group by only 4.88%. This indicated that the breeding heifers of the research group fed with the NaHum additive grew more intensively and could be assumed to reach the productive age sooner, thereby reducing the cost of herd renewal for the farm. This assumption was also confirmed by the relative growth ratio (RGR), which was higher for the research group than for the control group, starting from stage 2 of the research. It should be noted that at stage 3, when the breeding heifers were fed the highest dose of NaHum (0.6 mL/kg), the relative growth ratio for the research group was the highest, exceeding that for the control group by 9.3%.

Table 9. Costs of feed and the relative growth ratio

Parameters	Group		Change against control	P -value
	Control (n=21)	Research, NaHum (n=21)		
Starter feed, EUR/per animal/trial	222.37	229.17	+6.81	-
Milk, EUR/per animal/trial	140.08	138.32	-1.76	-
Hay, EUR/per animal/trial	0.23	0.23	0	-
NaHum, EUR/per animal/trial	-	23.55	+23.55	-
FC, total EUR/per animal/trial	362.68	391.28	+28.59	-
FC ADWG, EUR (formula 3)	13.99	14.67	+0.68	-
FC WG, EUR (formula 4)	2.80	2.93	+0.14	-
RGR1 (formula 5)	0.574^{Aa}±0.001	0.555^{Aa}±0.003	-0.019	0.431
RGR2, (formula 5)	0.428^{Ba}±0.001	0.429^{Ba}±0.001	+0.001	0.235
Difference; P-value, stage 1/stage 2	0.146; P=0.014	0.126; P=0.023	-	-
RGR3 (formula 5)	0.305^{Ca}±0.002	0.334^{Cb}±0.002	+0.028	0.001
Difference; P-value, stage, 1/stage 3	0.269; P=0.000	0.221; P=0.001	-	-
Difference; P-value, stage, 2/stage 3	0.123; P=0.001	0.095; P=0.000	-	-
RGR (formula 5)	0.436^{a}±0.003	0.439^{a}±0.001	+0.004	0.342

* FC—feed cost, BW—body weight, ADWG—average daily weight gain, WG—total weight gain. RGR—relative growth ratio per trial and period (RGR1—at stage 1; RGR2—at stage 2; RGR3—at stage 3). Data are presented as means ± SD—standard deviation; A, B, C—significant difference between stages within group; a, b—significant difference between the groups, P<0.05

3-4- Clinical and other Blood Parameters of the Breeding Heifers, an Analysis of The Parameters

The haematological parameters of the blood of the breeding heifers are presented in Table 10. At the end of the dietary research, the results of blood tests for the breeding heifers for the following parameters: WBC, RBC, LYM, MO, Hb and HCT had no significant differences between the groups (P>0.05). A higher Hb level and RBC count was found in the research (NaHum) group (Table 11).

Table 10. Effects of NaHum on the serum biochemical parameters of the breeding heifers

Parameters*	Group		P-value
	Control (n=21)	Research (NaHum) (n=21)	
GLU (mmol/L)	4.50 ±0.432	4.48 ±0.594	0.856
TC (mmol/L)	2.67 ±0.537	2.60 ±0.672	0.704
TP (g/L)	68.55 ±4.169	67.97 ±4.773	0.683
ALB (g/L)	40.67 ±2.648	41.29 ±1.790	0.396
ALP (U/L)	244.39 ±75.085	282.93 ±107.156	0.186
TRIG (mmol/L)	0.29 ±0.092	0.25 ±0.074	0.147
P (mmol/L)	2.79 ±0.289	2.78 ±0.442	0.948
Ca (mmol/L)	2.51 ±0.157	2.55 ±0.153	0.389
Mg (mmol/L)	1.01 ±0.309	0.96 ±0.147	0.741

*GLU- glucose, TC- total cholesterol, TP- total protein, ALB- albumin, ALP- alkaline phosphatase, TRIG- triglyceride, P- phosphorus, Ca- calcium, Mg- magnesium. Data are presented as means ± SD—standard deviation; N.A.—not applicable

Table 11. Haematological parameters at the end of the research for the heifers fed with different feed

Parameters*	Group		P-value
	Control (n=21)	Research (NaHum) (n=21)	
WBC (10^9/L)	10.7 ±2.214	9.92 ±1.596	0.209
RBC (10^{12}/L)	8.42 ±0.741	8.51 ±0.731	0.722
LYM (10^9/L)	5.76 ±1.836	4.88 ±1.667	0.116
MO (10^9/L)	0.57 ±0.306	0.49 ±0.310	0.429
Hb (g/dL)	11.04 ±0.686	11.20 ±0.699	0.474
HCT (%)	31.58 ±2.236	32.22 ±1.842	0.300

*WBC- white blood cell, RBC- red blood cell, LYM- lymphocytes, MO- monocytes, Hb- haemoglobin, HCT- haematocrit. Data are presented as means ± SD—standard deviation.

The parameters of serum metabolites are presented in Table 10. As shown in Table 10, no significant differences in blood biochemical parameters were observed between the groups. Feeding the NaHum additive to the breeding heifers led to a decrease in total cholesterol and triglycerides and an increase in Ca and showed an improvement in their general health.

3-5- Enumeration of Faecal LAB, Enterobacteriaceae, Coliforms and C. Perfringens Microbiota Tested

During the research, diarrhea was not observed clinically in the research heifers. At the end of the research, the *Lactobacillus* spp. count in faecal samples was steady, yet a significant difference was found between both groups, with the average ranging between 6.95 (control group) and 8.49 log CFU/g (research (NaHum) group). The highest value ($1.39x10^9$ CFU per g) was found in the research (NaHum) group sample, whereas the lowest was in the control group ($1.4x10^6$ CFU per g). The average count of *Enterobacteriaceae* was lower in the research (NaHum) group (6.54 log CFU/g) than in the control group (7.84 log CFU/g), and a significant difference was found between both groups. The highest value ($2.6x10^8$ CFU per g) was found in the control group sample, whereas the lowest was in the research (NaHum) group ($1.38x10^5$ CFU per g). An opposite trend was observed in the count of coliform bacteria. The count was slightly lower in the faeces of the breeding heifers of the control group than in those of the research (NaHum) group. Both the highest count ($6.9x10^7$ CFU per g) and the lowest count of coliform bacteria (3.09x104 CFU per g) were found in the breeding heifers of the research (NaHum) group. The *Lactobacillus* spp./coliform ratio showed significantly higher values for the research (NaHum) group (>1) than for the control group (Table 12).

Table 12. LAB, Enterobacteriaceae and C. perfringens counts in the heifers' faeces

Parameters	Group		P-value
	Control (n=21)	Research (NaHum) (n=21)	
Enterobacteriaceae	7.84 ±0.438	6.54±0.82	0.056
Lactobacillus spp.	6.95 ±0.473	8.49 ±0.34	0.021
Clostridium perfringens	2.18 ±0.15	1.18 ±0.87	0.632
Coliforms	7.02 ±0.331	7.09 ±1.07	0.418
Lactobacillus spp./coliform ratio	0.99	1.20	N.A.

* Mean values within a row differ significantly (P<0.05); data are presented as means ± SD—standard deviation; N.A.—not applicable

4- Discussions

4-1- Potential Benefits of NaHum in Animal Breeding

The results of the current research indicate that NaHum holds promise as a potential ingredient for use in animal breeding and dietary applications. In animal breeding, NaHum has been shown to improve growth performance and immune function in various species, including poultry, swine, and cattle. For example, it was found that the inclusion of NaHum in broiler diets increased the feed conversion ratio and weight gain while reducing mortality rates [59]. In addition to its potential benefits in animal breeding, NaHum has also been investigated as a dietary supplement for humans. Some studies have suggested that NaHum may have antioxidant and anti-inflammatory properties, which could potentially benefit human health. For instance, researchers found that sodium humate demonstrated both antioxidant and anti-inflammatory effects in vitro and in vivo [60]. In addition to its potential benefits in animal nutrition, NaHum has also been investigated for its potential applications in the fields of agriculture, environmental remediation, and water treatment [61, 62]. As such, NaHum is a versatile and promising material with a wide range of potential uses.

NaHum extraction is a promising approach for producing this valuable humic acid derivative. The use of an alkaline solution to extract NaHum is a relatively simple and environmentally friendly process that can be easily scaled up for industrial production, making it an attractive option for applications such as animal breeding and dietary supplements [63]. Additionally, NaHum production from freshwater sapropel at an industrial scale has real potential because the raw material is abundant and the extraction process is relatively simple and cost-effective [64]. Moreover, research where NaHum has been obtained from freshwater sapropel has shown high purity and quality, as well as beneficial properties such as antioxidant and antibacterial activity [60, 64]. These properties make NaHum produced from freshwater sapropel suitable for a wide range of applications in the food and agricultural industries, as well as in medicine and cosmetics. The chemical composition of NaHum depends on the source material from which it is extracted, with varying proportions of humic and fulvic acids, as well as other organic and inorganic compounds [65]. One potential source of NaHum is freshwater sapropel, which is rich in organic matter and minerals [66].

Separate studies have shown that NaHum extracted from different sources can have similar or equal properties as an animal feed additive. For example, a study conducted by Trckova et al. (2012) [67] found that NaHum extracted from peat had similar effects on growth and immune function in broiler chickens as NaHum extracted from lignite [68]. Additionally, NaHum has been shown to improve growth performance in various animal species, including pigs, chickens, and cattle [55, 69, 70]. These findings suggest that NaHum extracted from freshwater sapropel could potentially have effects similar to other sources of NaHum as a feed additive. However, the chemical properties and effectiveness of NaHum can vary depending on the source material from which it was extracted and the extraction methodology. Therefore, NaHum extracted from different sources may have varying levels of nutrients and other organic and inorganic compounds, such as fulvic and humic acids, which can also affect its efficacy as an animal feed additive. However, while NaHum extracted from freshwater sapropel shows promise as a potential source of feed additive. In the current research the mineral composition of NaHum is similar (or identical) to the NaHum derived from other sources the mineral composition of NaHum reflected that reported in the scientific literature [71]. Further research studies are needed to assess its chemical properties and effectiveness in comparison with other sources of NaHum.

4-2- Changes in Growth Indicators of Breeding Heifers

In our research, the chemical composition of the starter feed (Table 3) was consistent with that used in dietary experiments with NaHum additives on heifers conducted by Wang et al. (2022) [18] and Raketsky et al. (2021) [72]. Raketsky et al. (2021) [72] and Crowe et al. (2018) [73] emphasized that heifers mature faster than bulls; therefore, to avoid excess fat formation, which is often the reason for culling heifers, they need a specially balanced diet and more careful monitoring. Research studies have shown that minerals and vitamins of natural origin included in animal feed can contribute to a higher live weight gain [74]. One such natural source of vitamins and minerals is sapropel; its chemical composition suggests that it could be used as a feed additive for cattle [75]. Yuca and Gul (2021) [76] found that inclusion of humate in the diet for Swiss Brown dairy cows (75 g; 100 g) during the prenatal and reproductive periods (from prepartum 40th to post-partum 60th day) had a positive effect on the composition of colostrum, milk yield, and health indicators. Kholif et al. (2021) [77] arrived at a similar conclusion in their research on early lactating Friesian cows.

In several research studies, it is mentioned that the growth rate of breeding heifers is essential for reproduction of the cow herd, as it accelerates the onset of puberty in breeding heifers and the possibility of insemination at an earlier age which, after calving, results in additional milk production and therefore contributes to food security, incomes, and employment. During our dietary research, the breeding heifers were fed according to the strategy developed on the farm of starting artificial insemination of heifers at 12 months [55]. In our research, the breeding heifers of the research group achieved a significantly higher live weight gain at the final stage, receiving NaHum at an intake rate of 0.6 mL/kg of live weight; this confirmed the finding of Radchikov et al. (2021) that the highest live weight gain was achieved while feeding heifers 0.6 mL of NaHum per kg of live weight [55]. The greater increase in live weight in the breeding heifers NaHum group is explained by the fact that sapropel and humic substances improve the animals' appetite, and the smell

of sapropel promotes the animals' willingness to eat it. Accordingly, this explains the higher consumption of starter feed in the research group and the higher live weight gain. The results indicated a higher growth rate of the breeding calves, reaching a live weight of more than 150 kg in 110 days—155.93±15.63 kg in the control group and 156.4±17.54 kg in the research group. Such a live weight significantly exceeded the average live weight of heifers at 4 months (125-136 kg) found by Costa et al. (2021) [17], which indicated a faster growth rate influence of NaHum. Research studies on humic substances added to drinking water for animals have reported an increase in live weight gain for various species of productive animals, including heifers [72], which was consistent with the findings of the present research. In addition, de Lourdes Angeles et al. (2022) indicated an improvement in the welfare of heifers and a decrease in the incidence of diarrhea in heifers [78]. The present research on the NaHum additive included in the diet for breeding heifers found that the breeding heifers displayed an improvement in their welfare, activity, and coat condition, which was fully consistent with the findings made by Wang et al. (2022) and De Lourdes Angeles et al. (2022) [18, 78].

4-3- General Health Status of the Breeding Heifers

Haematological and biochemical tests on the blood of farm animals are necessary to obtain information about their ongoing processes and general physiological state of health. Some research studies have found that humic substances derived from sapropel stimulate haematopoiesis and improve metabolism and immunity [55]; sodium humate possesses antidiarrheal, antimicrobial, anti-inflammatory, and growth-promoting properties, as well [79]. Overall, the blood test parameters of the breeding heifers tested were within the reference intervals [80, 81]. Haematological parameters such as the WBC, LYM, RBC count and Hb concentration are used to determine the health status of animals [82–84]. The present research found that the average WBC counts in the control and the research (NaHum) groups were 10.7 and 9.92 (109/L), respectively, and the LYM counts were 5.76 and 4.88 (109/L), respectively. Since the count of leukocytes and lymphocytes usually increases in cases of various diseases, it could be concluded that NaHum additives provided breeding heifers with better protection against various inflammatory diseases. The RBC count and the Hb level found in the blood of the heifers were also within the normal intervals [80, 81]; however, both parameters found in the research (NaHum) group were higher than those in the control group (by 1.06% and 1.43%, respectively), which indicated that the NaHum additive encouraged heifers to ingest more bulk feed containing more iron than milk [85].

The findings of the present research indicate that the NaHum additive did not cause significant changes in serum metabolite parameters. All the parameters tested for the breeding heifers of the research group were within the normal intervals. The present research found a higher level of ALP (on average by 13.6%) in the research (NaHum) group than in the control group, which might indicate a more stimulating effect of the additive on bone formation, as well as a stimulating effect on intestinal permeability.

The purpose of including any feed additive in the animals' diet is to improve feed digestibility, thereby promoting their development and weight gain. It is equally important to provide modulation of the gut microbiota to reduce the incidence of diseases and improve growth performance. There are reports by several researchers on the modulatory effects of NaHum on the microbiota population [86, 87]. The present research proved that daily intake of the NaHum additive at a rate of 60 mL per 100 kg of body weight by the breeding heifers reduced the count of *Enterobacteriaceae* (average 1.6×107 CFU/g) and *C. perfringens* (average 49 CFU/g) in the intestinal tract, compared with the control group (average 1.11×108 and 1.6×102 CFU/g, respectively), yet it significantly increased (on average by 18.1%) the count of *Lactobacillus* spp. (on average 1.31×107 CFU/g in the control group and 4.97×108 CFU/g in the research (NaHum) group. The effect of the NaHum additive used in dietary research on an increase in the count of *Lactobacillus* is considered significant because their transient multiplication in the digestive tract *in vivo* provides a microbial barrier against the development of pathogenic bacteria [88, 89]. The *Lactobacillus*/coliforms average ratio was 1.20 for the breeding heifers of the research group (NaHum), while for the control group heifers it was 0.99, which indicates the ability of the NaHum additive to modulate the microbiota of the intestinal tract of the heifers.

4-4- The Limitations of the Study and Prospects for Future Research

In the future, investments in research on the food system "Farm to Fork" are also needed to make healthy food available to the population; in addition, the solutions should be local, as this would increase revenue for businesses involved in the food supply chain, as well as employment opportunities in rural areas [90, 91]. The present research significantly adds to the knowledge about feed additives that are derived from freshwater lake sapropel and included in diets for Holstein breed heifers. In the dairy industry, it is important to improve the principles of feeding by using various additives and premixes, thereby supplying the necessary minerals and biologically active principles for the development of the heifer's organism and, consequently, improving the health of the animals, stimulating their growth, and favourably preparing the animal organism for the reproduction stage.

Therefore, future potential research priorities could include the following: 1) continuing research on NaHum consumption and seeking optimal doses for older heifers to determine whether the positive effect from NaHum consumption persists in subsequent growth periods; 2) continuing research on cows (after the birth of their calves) to

establish whether the positive results of the present research for the early growth stage of breeding heifers also continue in the productivity and health of the cows; 3) examining how inclusion of the NaHum additive in diets for breeding heifers affects various emissions from livestock production; 4) conducting in-depth research on how the use of such an additive affects the health and longevity of breeding heifers, thereby increasing output while consuming fewer resources; 5) seeking ways to extract the dry fraction of NaHum derived from freshwater sapropel to provide a simpler possibility of dosing NaHum in the livestock feeding process; 6) assessing the chemical properties and effectiveness of NaHum extracted from freshwater sapropel in comparison with other sources of NaHum.

5- Conclusion

In examining the composition and chemical properties of freshwater sapropel extracted in Latvia, no components harmful to animals were found, meaning it could be ingested by farm animals. To reduce the ash content of the additive, NaHum was produced by adding NaOH to sapropel under laboratory conditions. All the replications were done in Latvia (the temperate climate zone) in the wettest and coldest climatic conditions, i.e., in autumn, winter, and spring. Therefore, it could be assumed that when conducting such research in the warm period of the year, the results would probably be even more favorable because less of the ingested nutrients would be used by the heifers for thermoregulation of the body.

The research results suggest the following: 1) NaHum extracted from freshwater sapropel is a promising and versatile material with potential in animal breeding and dietary supplements; the extraction is a relatively simple and environmentally friendly process that can be easily scaled up for industrial production; 2) The consumption of starter feed by the research group breeding heifers was significantly higher at stage 1 at 0.874 kg/d and at stage 3 at 5.665 kg/d, which were respectively 0.119 and 0.444 kg/d more than in the control group. This could explain the higher live weight gain of the research group heifers; 3) The inclusion of NaHum in the diet for breeding heifers at an intake rate of 0.6 mL/kg of live weight increased the live weight gain of the research group's heifers on average by 47.98 kg, which was 4.37 kg more than in the control group ($P<0.05$); 4) An increase in daily live weight gain at all the stages of the research was found in the research group heifers, from 1.144 kg at stage 1 to 1.371 kg at stage 3. During the entire research period, the average live weight of the breeding heifers of this group increased by 1.277 kg per day, which was 35 g more than in the control group; 5) The inclusion of NaHum in the diet for breeding heifers did not significantly affect the haematological and biochemical parameters of blood of the heifers, while changes in the counts of faecal microbiota (increase in lactobacilli between 6.95 (control group) and 8.49 log CFU/g (research (NaHum) group, $P<0.05$) and a decrease in the counts of enterobacteria and clostridia ($P>0.05$) indicated the ability of humates to provide the heifers with immune status and modulate their intestinal microbiota; 6)The research hypothesis proved to be true because feeding the NaHum additive derived from freshwater lake sapropel to the breeding Holstein heifers up to 5 months of age increased their feed intake and live weight gain, as well as activity and overall health.

The management of the farm is recommended to continue research on both older heifers (until the calving period) and cows by applying the methodology developed by the present research. Opportunities should be sought to commercialize the findings of the present research. In addition, further research is needed to assess the chemical properties and effectiveness of sapropel-derived NaHum in comparison with NaHum from other sources.

6- Declarations

6-1- Author Contributions

Conceptualization, D.K., A.V., I.P., and L.P.; methodology, D.K., A.V., I.V., L.P., and I.P.; validation, D.K., A.V., L.P., and I.P.; investigation, D.K., A.V., I.V., D.G., G.G., L.P., S.M., and I.P.; resources, I.P.; writing—original draft preparation, D.K., A.V., I.V., L.P., and I.P.; writing—review and editing, D.K., A.V., I.V., L.P., and I.P.; visualization, L.P. and I.P.; supervision, I.P.; project administration, L.P.; funding acquisition, I.P. All authors have read and agreed to the published version of the manuscript.

6-2- Data Availability Statement

The data presented in this study are available on request from the corresponding author.

6-3- Funding

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6-5- Institutional Review Board Statement

The present research complied with the requirements of the Law of the Republic of Latvia (RoL) (2007) "On the European Convention for the Protection of Animals Kept for Farming Purposes and the Protocol". The research was carried out in cooperation with an agricultural enterprise, Ogres piens Ltd, in its livestock shelter in Latvia, Ogre Municipality, Ogresgals Parish (address: Ogre, LV-5041, Vidzeme region), where the NaHum additive derived from freshwater sapropel was fed to breeding Holstein heifers under production conditions. Observations and data were recorded and blood samples were collected during routine work on the farm, and, according to EU Directive 2010/63/EU, Article 1, Paragraph 5, Point a, approval from the ethics commission for non-experimental agricultural practices was not required.

6-6- Informed Consent Statement

Not applicable.

6-7- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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