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The Possibilities of the Application of Feed Additives from Macroalgae in Sustainable Mineral Animal Feeding

[1]Izabela Michalak, [1]Katarzyna Chojnacka and [2]Peter Glavič
[1]Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Wrocław, Poland
[2]Faculty of Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia

Abstract: Problem statement: The idea of the application of biological material as mineral feed additives could constitute an innovative practice that would encourage environmental sustainability. The main idea of this study was to present the advantages of macroalgae enriched with microelements when compared to inorganic salts. **Approach:** In order to evaluate the potential participation of macroalgae in sustainable animal feeding, it was necessary to consider several concepts, such as: Waste Minimization (WM), Cleaner Production (CP) and Pollution Prevention (PP), which were closely related to sustainable production. Special attention was also paid to the prevention of waste generation during production process of mineral feed additives from macroalgae. **Results:** This kind of feed additives could contribute to the minimization of nutrient excretion by animals, by optimizing nutrient availability and proportion in the animal diet. **Conclusion:** The application of macroalgae in animal feeding could be considered as preventive environmental strategy, which would reduce the risks of the excess of microelements in the environment and in the animal diet.

Key words: Sustainable development, macroalgae, mineral feed additives, animal feeding, sustainable animal feeding

INTRODUCTION

The notion of sustainability in animal feeding has become a popular topic. According to the "Sustainable European Farm Animal Breeding and Reproduction" (SEFABAR) project, there are five criteria for sustainable animal breeding and reproduction: (1) Quality, which aims at improving product quality, health and welfare of animals and food safety for consumers, (2) Diversity, at maintaining biodiversity, improving adaptability to diverse environments, improving product diversification, (3) Acceptability, at fulfilling ethical and animal welfare standards of production, (4) Environment, at minimizing pollution, improving efficiency of feed resource and land utilization, (5) Economics, at improving production efficiency and economic viability, both short and long term[1].

In this study, three criteria with reference to sustainability were considered: the quality, the environment and economics. Animal feeding has influence on food quality. It focuses on feedstuff (composition, nutrient quality, hygienic quality), on energetic and nutrient requirements of the different animal species as well as on feed additives. Apart from the importance concerning animal feeding, the health of animals also plays an important role for the quality of food and other animal products influencing human health. The attention to the nutrient content of animal diet would help to maintain healthy animals, environment and reduce feeding costs. Nowadays, it is very important to maintain a balance of nutrients on the farm. Producers should take into account all nutrients coming into and leaving the farm. Nutrient inputs in the form of feed, fertilizers must balance outputs such as crops, manure and animals for market. An imbalance results in soil contamination and a loss to the environment[2]. The goal of efficient and productive feeding of animals, within economic and environmental constraints, is to provide essential available nutrients for maintenance and production with minimal excess.

MATERIALS AND METHODS

In order to evaluate the potential participation of macroalgae in sustainable animal feeding, several dozen of publications were analyzed. For the analysis, green macroalge were chosen, since they are abundant in the Baltic Sea. The procedure of biosorption

Corresponding Author: Izabela Michalak, Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Wrocław, Poland

experiments of microelement ions by macroalgae was described elsewhere[13,14].

The economic analysis was prepared under the cost plus method of pricing, which is based on full cost accounting model. Only direct and indirect costs were included in product cost calculation (excluding general (overhead) costs).

RESULTS AND DISCUSSION

Mineral feed additives in animal breeding: The current, intensive animal production requires the application of different types of feed additives. Among them, the following kinds can be distinguished: Vitamins, provitamins, trace elements, antibiotics, probiotics, antioxidants, food coloring agents, preservatives, herbs, acidity regulators, emulgent substances, aromatic and appetizer substances and kokcydiostatics[3]. All of them play a significant role in the proper nutrition of animals, but in the recent years, an increasing attention has been paid to the supplementation of animal diet with sufficient quantities of microelements. Increased growth rates and milk/meat/eggs production greatly increase requirements for minerals. Nowadays, mineral deficiencies and imbalances for animals are reported from almost all regions of the world. For many classes of livestock, including swine, poultry, feedlot cattle and dairy cows, mineral supplements are incorporated into diets, which generally assure that animals receive required minerals, but there is a considerable difference in the availability of microelements from different sources[4]. At present, microelements are supplied to fodder mainly as inorganic salts[5], which are characterized by low bioavailability to animals. In connection with this, microelements possess transit character and can cause environmental contamination. It is also important to indicate, that microelements, which are essential to animals, are simultaneously heavy metals (e.g., zinc(II), copper(II), chromium(III)), which can be dangerous in higher concentrations. In the recent years, there has been also an increasing interest in the application of organic trace minerals, which are considered to be more available than inorganic forms and more similar to the forms that occur in food, feed and also in the body[5]. The bioavailability of microelements from organic forms is 10 times higher than from inorganic forms[6]. Their main disadvantage is high price[4]. Therefore, there is a need to elaborate such mineral feed additives, which would supply minerals in quantities adjusted to the animal requirements and moreover in a non-toxic and highly bio-available form. Recently, a special attention has been also paid to the resource sufficiency, which presumes that a production practice is sustainable if the resources needed to carry on the practice are available or foreseen to be available in the future. The debate over sustainability as resource sufficiency has tended to be risen with respect to elasticity for resource substitution[7].

Macroalgae as mineral feed additives in animal diet: In this study, the application of macroalgae as mineral feed additives is proposed, aiming to increase the bioavailability of microelements from feed additives of biological origin, thereby increasing the microelement content in animal products (meat, eggs, milk) and moreover to improve the feed value and finally to increase the livestock productivity. The usage of macroalgae as feed additives is an inventive application of biotechnology in animal nutrition. Provision of animal feeds with enhanced nutritional quality improves the sustainability of animal production[8]. All materials of organic origin are of major importance to sustainable development, simply because they can be grown and are renewable, as opposed to inorganic materials e.g., minerals[9]. A special attention should be paid to algae, because two-thirds of the biomass on the Earth consists of over 25,000 species of algae. They are found in all climatic zones and are considered as renewable source of nutrients and minerals[10]. Moreover, in the literature it is suggested, that biological material, such as aquatic plants[11,12] or algae[13-15] could be used as a carrier of microelements in animal feeding. Fodders of plant origin are known to be poor in microelements. Algae possess a unique property of binding minerals from aqueous solutions via biosorption process, which is not metabolically controlled and describes passive binding of metal ions to non-living biomass[16]. The application of this process gives the opportunity to increase the concentration of microelements not of several dozen percent, but several hundred or even thousand percent (for example: The concentration of Cu(II) ions in natural biomass of *Pithophora varia* Wille was 37.5 mg kg^{-1} and after biosorption 2,952 mg kg^{-1}, which gives an increase of 79 times[14]).

There are two possible methods of enriching biomass with microelements-single-and multi-metal system. Both systems have advantages and disadvantages. The single-metal system is easier to control, model and predict, yields higher sorption capacities for a given ion, but on the other hand it is more complicated to carry out this process on industrial scale. In the case of multi-metal system, the biomass could be enriched with all microelements at the same time, but the efficiency of enrichment process is lower than in single-metal system, as a result of competition of metal cations for binding sites on cell surface. General scheme of the production of mineral feed additives from macroalgae (containing Cu(II), Zn(II), Co(II), Cr(III) and Mn(II) ions) in single-and multi-metal system is showed in Fig. 1.

Out of all sources of microelements, the application of macroalgae as mineral feed additives could constitute environmentally friendly and economically beneficial solution. Moreover, macroalgae are approved for human and animal consumption by the obligatory law[17,18]. Contrary to algae, inorganic salts-the most common source of minerals in fodders, are non-renewable sources of microelements. Although the biomass of algae is enriched with inorganic salts, the bioavailability of this organic form will be probably higher than the salts served in its inorganic form. Therefore, there will be a necessity to add smaller amounts of biological feed additives and to establish new standards for such preparations. The shift from non-renewable materials to renewable bio-materials is a central concern for some industrial ecologists. It is also important to indicate, that macroalgae could be used not only as a source of minerals, but also as a source of lipids, proteins, vitamins and carotenoid pigments[19-23].

The advantages of the application of macroalgae as feed additives: Since the animal is the initial source of nutrient excretions and odors from its operations, diet manipulation is a practical and potentially economical way to control excess nutrient excretions and odor emissions that will have a major impact to minimize pollution of water, soil and air[24]. The application of macroalgae in mineral animal feeding could significantly contribute to the minimization of nutrient excretion by animals. In the recent years, the trend toward intensive animal production has raised concerns about concentrated production of manure. These concerns challenge producers to adopt not only improved manure management methods, but also methods for reducing the content of nutrients, which are supplied to feeds in excess in comparison with the given dietary requirements of animals. Diets are often over-supplemented to provide a margin of safety (for example to compensate for the variable composition and digestibility of feed sources)[2]. Very often the content of nutrients in manure reflects their concentrations in the feeds consumed and the degree of feed conversion by the animals[25]. This practice should be avoided, because any nutrient fed in excess is excreted and contributes to the buildup of nutrients in the soil. Therefore, there is a need to decrease elements concentrations in manure.

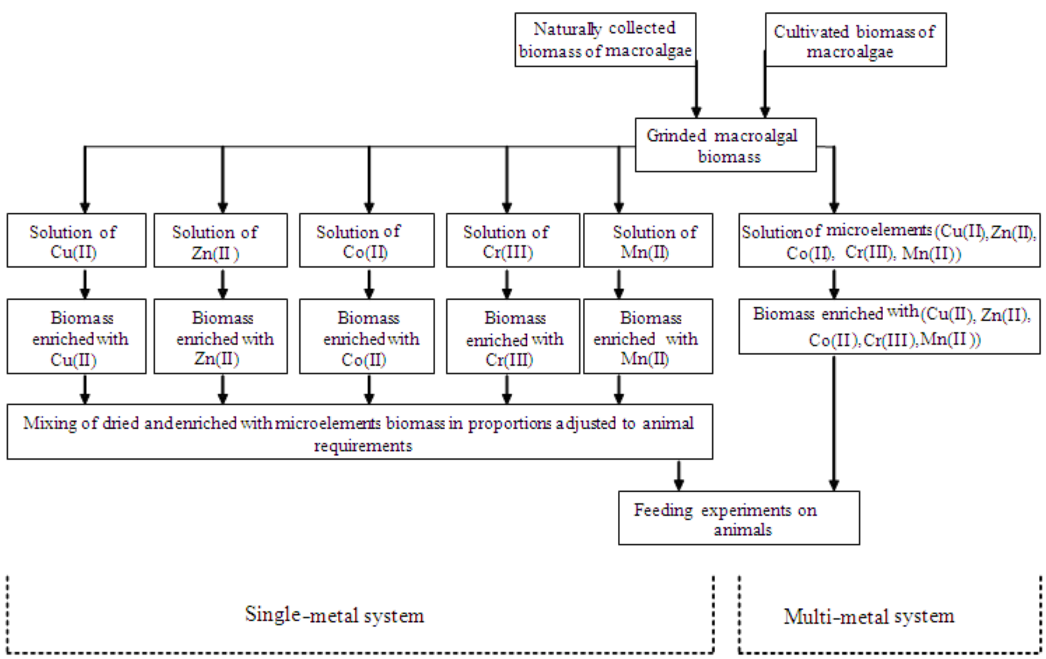

Fig. 1: General scheme of microelements biosorption by dried macroalgae in single- and multi-metal system (own work)

Optimizing nutrient availability and proportion in the diet to meet the animal's maintenance and production requirements is a recognized practice to decrease nutrient excretion[24]. The higher the bioavailability of a particular nutrient in a feed ingredient, the less feed will be required and the less will be wasted. In the literature it is suggested, that for example, application of phytase-containing transgenic seeds as additives in animal feed would help to reduce phosphorus level in excrements[26]. The most optimal way to reduce another macroelement-nitrogen in excreta is to lower the content of crude protein fed and to supplement diets with synthetic amino acids. In the case of microelement, as it was mentioned above, usage of organic forms of Cu, Fe, Mn and Zn in swine diets resulted in lower levels of these minerals added to the diet and excreted if compared to conventional dietary mineral sources[24].

In the literature it is also reported, that yeasts enriched with Se(IV) and Zn(II)-*Saccharomyces cerevisiae* were more available to laying hens than inorganic forms (sodium selenite and zinc oxide). The content of Zn(II) ions in eggs was 2.0% higher in the case of application of enriched with Zn(II) ions yeasts than in the case of zinc oxide[27]. Also the content of Se(IV) in milk and blood of cows was higher 65 and 36%, respectively in the case of feeding animals with enriched yeasts than with inorganic form-Na_2SeO_3[28]. On this basis, it can be assumed that macroalgal biomass enriched with microelements (Cu(II), Zn(II), Co(II), Cr(III), Mn(II)) would have higher bioavailability to animals than inorganic forms of these microelements. This hypothesis was confirmed in feeding experiments carried out on laying hens. The results showed that microelements from enriched macroalgae were more bio-available to hens than inorganic salts from fodder in the control group and moreover were transferred in higher quantities to egg yolk, white and blood[29]. Therefore, enriched macroalgal biomass could be employed not only in the supplementation of livestock diet with the recommended daily intake of some macroelements and trace elements, but also in the biofortification of eggs or meat with microelements. This kind of biofortified products could be applied as a new type of functional food, which would supplement microelements deficiencies in human diet as food, not as mineral salts.

It is also important to emphasize, that animal manure, which is rich in N and P could be converted in to algal biomass. Mulbry *et al*.[30] suggested growing algae on N and P present in manure. The volume of algal biomass from 100 dairy cows would provide available N fertilizer equivalent for 6 ha of corn at 150 kg ha^{-1}. As P fertilizer used for amending soils at 100 kg P ha^{-1} this volume of algal biomass would support 4 ha of cultivation[30].

Improvement of the biosorption process by macroalgae: Generating significant amounts of waste is not sustainable for today's society. Nowadays, there are many concepts, which aim at reducing waste generation, minimizing the impact of chemicals and chemical processes on the environment and the public and minimizing any hazards to the worker. One such a concept is Green Chemistry, which concerns the design of chemical products and chemical processes that reduce or eliminate the use and/or generation of hazardous substances[31]. According to the first principle of Green Chemistry-prevention of waste creation, it is better to prevent waste than to treat or clean them up after their generation[32]. In the production process of mineral feed additives from macroalgae, several stages could be improved. The whole enrichment process consists of the following main steps. At first, a reaction mixture of a given metal or metal ions (using inorganic salts that are commonly used as the source of micronutrients in animal feeding), with adjusted pH is pumped to a biosorber (multi-metal system) or biosorbers (single-metal system), where metal ions become bound to the biomass (Fig. 2). Then, suspension with algal cells is pumped to separation unit (e.g., sedimentation tank), where separation takes place. The biomass is collected as sludge from the bottom and moved to a dryer.

The main disadvantage of this process is generation of large volumes of microelement solutions after process. The main idea is to recycle these solutions and to select appropriate genus of macroalgae, which would be characterized by high biosorption capacity. Biosorption capacity is expressed by the mass ratio of a given microelement bound by the dry biomass [in mg g^{-1}]. As a result, the concentration of microelement ions in waste stream would be lower and additionally the operation costs of a treatment plant and waste disposal cost would be reduced to a minimum. This approach would lower the volume of water used and also the costs of purchasing fresh water. Today there is a world-wide shift toward encouraging in-plant water conservation, recycle and reuse. Additionally, waste streams generated in this process (with known composition) could also undergo treatment by macroalgae, which finally could be applied as mineral fertilizers and soil conditioners.

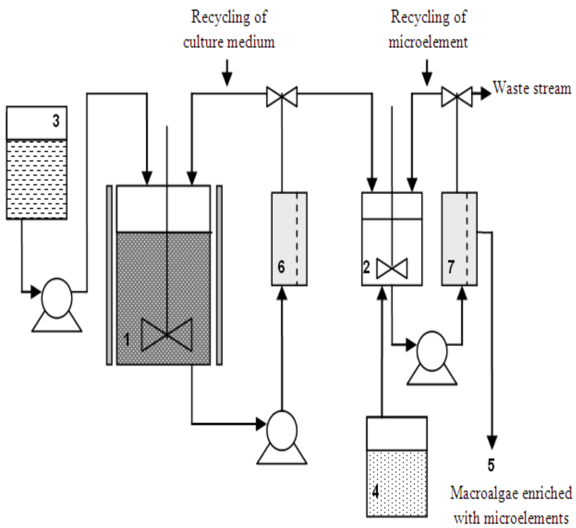

Fig. 2: General scheme of the production of mineral feed additives from macroalgae (own work) (1): Cultivation of macroalgea, (2): Biosorber, (3): Container with culture medium, (4): Inorganic salts, (5): Drying and storage of enriched algae, (6 and 7): Separation units

Economic evaluation of the production process of mineral feed additives from macroalgae:

Introduction: In order to perform a general economic evaluation of the production process of mineral feed additives from macroalgae, several assumptions have been made. They concerned mainly: The biomass collection and the production process and are presented below:

The biomass collection:

- Biological material (macroalgae) from marine resources (the Baltic Sea)
- Marine macroalgae will be taken out from the sea water
- Wet material will be cleaned and dried
- Processed material will be delivered to the production by the marine factory
- Planned production of dry biomass equals to 100 kg d^{-1}

The production process:

- It is assumed that the production process will be based on natural, dry biomass
- The production process will include: Cleaning, enriching the dried biomass of macroalgae with microelement ions (Cu(II), Zn(II), Mn(II) and Co(II)), drying the biomass after biosorption process, packaging and storing
- The obtained product will be feed additive
- The continuous production will be assumed
- Just-In-Time (JIT) production process
- Planned cost of in-coming (material) and out-going (product) transport equals zero (supplier of materials covers all the transport costs to the gate of the company and purchaser of the products covers all the costs of transport [on gate product delivery])

On the basis of the mentioned assumptions, the equation for the calculation of the Total annual cost of production (C_{TP}) was proposed (1). C_{TP} included the direct and indirect costs:

Total annual cost of production (C_{TP}) = direct costs (C_D) + indirect costs (C_I)

$$C_{TP} = C_D + C_I \tag{1}$$

In order to calculate the direct and indirect costs, the following factors were taken into consideration:

- The direct costs include the following: Costs of materials purchase; costs of the main production, e.g., cleaning; enrichment process; drying; packing (confection of the product); storing in the stage of pre-and after-production process will be minimized because of the JIT production process; direct costs of wages; other direct costs
- The indirect costs include the following: Depreciation costs (real estate, machines, equipment); indirect costs of wages; costs of energy, water and other energy elements connected with the production process; costs of machinery and equipment maintenance; other indirect costs

Calculations:

Biosorption process: In order to enrich the biomass of marine macroalgae (e.g., *Enteromorpha prolifera*) with the following microelement ions (Cu(II), Zn(II), Mn(II) and Co(II)), the biosorption process was performed in the containers (V_i = 40 l), which were filled up with the microelement ions solutions (the mass concentration of each microelement ion was assumed to be 300 mg L^{-1}). During one biosorption process, 0.04 kg of the biomass was enriched (to obtain 1 kg of the enriched biomass, 1,000 l of the aqueous solution of microelement ion will be needed).

In the Table 1, the average specific price of mineral salt (in EURO kg^{-1}, it was assumed that one EURO equals four PLN), which was used in biosorption process, is presented. Taking into account the mass of mineral salt, which is needed to prepare the microelement solution with the mass concentration of 300 mg L^{-1} ($CuSO_4 \cdot 5H_2O$-47.2 g; $ZnSO_4 \cdot 7H_2O$-52.8 g; $MnSO_4 \cdot H_2O$-39.6; $Co(NO_3)_2 \cdot 6H_2O$-59.3 g), the number of biosorption cycles, which were performed per unit mass of the mineral salt was calculated. On this basis the average specific price of the enriched biomass (EURO) was evaluated.

Costs plan per month (30 days): It was assumed, that the cost of the material (biomass) purchase equals 25 EURO kg^{-1}. All of the calculations presented in Table 2 were prepared only on the presumption, not on real data.

Taking into consideration the information from Table 2, the total annual cost of production (not including general costs of company) is 212,500 EURO a^{-1} (C_{TP} = 200,000 EURO a^{-1} + 12,500 EURO a^{-1}).

Analysis: The economic analysis shall provide the information of specific cost of dry biomass treated as a final product-mineral feed additive. Taking into consideration the above-mentioned the specific cost shall be valued at C_{TP} = 212,500 EURO/(2 850 kg) = 74.5 EURO kg^{-1}. Presuming the margin, which shall also cover the general expenses, on the level of 50% of C_{TP}, the proposed total specific cost of the product shall be valued at 112 EURO kg^{-1}.

Table 1: The average specific price of mineral salt (in EURO) used in the biosorption process

Mineral salt	Specific price of mineral salt (EURO kg^{-1})	The mass of the enriched biomass from prepared 1 kg of mineral salt*	The average specific price of 1 kg of the enriched biomass (EURO kg^{-1})
$CuSO_4 \cdot 5H_2O$	15.1	0.84	18.0
$ZnSO_4 \cdot 7H_2O$	11.5	0.72	16.0
$MnSO_4 \cdot H_2O$	48.3	1.00	48.3
$Co(NO_3)_2 \cdot 6H_2O$	29.0	0.64	45.5

*: e.g., 1 kg of Cu(II) salt costs 15.1 EURO. To prepare one solution of Cu(II) ions with the concentration 300 mg L^{-1}, 47.2 g of salt is needed. This means that from one kg of salt, it is possible to conduct 21 biosorption processes. In one process, 0.04 kg of the biomass is enriched (from 1 kg of salt it is possible to obtain 0.84 kg (0.04×21) of enriched with Cu(II) ions biomass). This means that one kg of biomass enriched with Cu(II) ions will cost approximately 18 EURO

Table 2: Costs plan per month

	Type	Mass/Mass fraction/Persons A	Planed value in EURO B	Notice
1.	**Direct costs**	---	~200,000	1
1.1	Costs of materials purchase	3,000 kg	75,000	
1.2	Costs of main production	---	113,522	
1.2.1	Dry cleaning	5% of 1.1 B	3,750	
1.2.2	Enrichment with Cu, Zn, Mn, Co		91,022	2
1.2.3	Drying	20% of 1.1 B	15,000	
1.2.4	Packing	5% of 1.1 B	3,750	
1.3	Storing	2% of 1.1 B	1,500	
1.4	Direct costs of wages	3 persons	2,250	3
1.5	Other direct costs	10% of (1.1 B+1.4 B)	7,725	
2	**Indirect costs**	---	~12,500	4
2.1	Depreciation costs of real estate	1 building of the initial value of 120,000 EURO	250	5
2.2	Depreciation costs of machinery and equipment	Initial value of machinery and equipment of 125,000 EURO	2,250	6
2.3	Indirect costs of wages	½ of 1.4 B	1,125	
2.4	Costs of energy, water and other energy elements	3% of 1 B	6,000	
2.5	Costs of machinery and equipment maintenance	30% of 2.2 B	675	
2.6	Other indirect costs	1% of 1 B	2,000	

Notice description: (1): The detailed annual cost is 199,997 EURO a^{-1}. For further analysis the 200,000 EURO a^{-1} is taken into consideration, (2): It was assumed that the planned loss of material in the production process equals 5%. Further analysis is based on 2 850 kg (95% of the purchased material). If we assume, that we want to obtain equal masses of the biomass enriched in four microelement ions, we will have 712.5 kg of the biomass for each microelement. On the basis of data presented in Table 1, the production of the biomass enriched with Cu(II) will cost 12,825 (EURO/712.5 kg), with Zn(II)–11,400 (EURO/712.5 kg), Mn(II)–34,378 (EURO /712.5 kg) and Co(II) –32,419 (EURO /712.5 kg). The total sum equals 91,022 (EURO), (3): It was assumed that to carry out the production process, 3 persons are needed for 8 h d^{-1} (one shift only, gross) [3×750 EURO = 2,250 EURO], (4): The detailed cost is 12,300 EURO. For further analysis 12,500 EURO is taken into consideration, (5): The initial value of the building is 120,000 EURO. The depreciation is planed for 40 years that is 2.5% a^{-1}, which equals per month 250 EURO m^{-1} (120,000 × 2.5% = 3,000×1/12 = 250 EURO m^{-1}), (6): The initial investment of the machinery and equipment is 135,000 EURO. The depreciation is planned for 5 a, e.g., 20% a^{-1}, which equals 2,250 EURO m^{-1} (135,000×20%×1/12 = 2,250 EURO m^{-1})

Table 3: Standards in Animal Feeding, mass (g) of enriched alga in single-metal systems added to 1 kg of feed to cover 100% of upper requirement in laying hens and swine diet, respectively and the mass of feed (kg), which could be prepared from 1 kg of the enriched biomass

Micro-element	q_{max} (mg g^{-1})[33]	The requirements for microelement in feed (mg kg^{-1})		The mass fraction of enriched *E. prolifera* introduced to the feed (g kg^{-1})		The mass of feed (kg), which could be prepared from 1 kg of the enriched biomass	
		Laying hens[34]	Swine[35]	Laying hens	Swine	Laying hens	Swine
Cu(II)	54.0	5-6	20-165	0.111	3.0600	9,009	327.000
Mn(II)	23.6	60-70	30-40	2.970	1.6900	337.000	592.000
Zn(II)	59.5	50-60	70-150	1.010	2.5200	990.000	397.000
Co(II)	41.9	-	0-0.5	-	0.0119	-	84,034

Conclusions for the general economic evaluation: Although the average specific price of one feed additive from macroalgae is relatively high, this kind of feed supplement might be very valuable from the nutritional point of view for animals.

In our study, we confirmed that enriched macroalgae are more bio-available to animals than inorganic salts. The results obtained from feeding experiments on laying hens are promising. Supplementing of bio–metallic feed additives to the diet of laying hens resulted in higher microelement transfer to eggs and enhanced the color of yolk. It was also found that the presence of enriched with microelements *Enteromorpha prolifera* and *Cladophora* sp. in laying hens diet influenced advantageously eggs weight, eggshell thickness as well as body weight of hens[29].

It is worth pointing out, that from 1 kg of enriched with Cu(II) biomass, we are able to prepare 9,009 kg of feed for laying hens and 327 kg for swine. The calculation for the remaining microelements is presented in Table 3. For the calculation, maximum biosorption capacity of the biomass (q_{max}) and the requirements of the animals for given microelement were taken into consideration. We assumed that the upper levels of the requirements for microelements will be covered by enriched macroalgae.

CONCLUSION

The main goal of the novel option presented in this study is to follow the principles of Pollution prevention, which aims at stopping the pollution before it is generated and at achieving sustainable improvements, involving not only conservation of natural resources and materials, but also preventing accidental releases and avoiding exposure to the toxic and dangerous materials. The application of macroalgae in animal feeding could be considered as preventive environmental strategy, which would reduce the risks of the excess of microelements in the environment and in the animal diet. Therefore, feed additives from macroalgae could participate in sustainable mineral animal feeding. Although the average specific price of one feed additive from macroalgae is relatively high, this kind of feed supplement might be very valuable from the nutritional point of view for animals.

Nevertheless, it is important to point out, that substantial difficulties could exist in replacing inorganic feed additives by more bioavailable form, e.g. macroalgae enriched with microelements. This would depend on the natural sources of raw biomass and moving the process from laboratory to industrial scale. This type of change would involve long term strategic decisions. Nowadays, more companies take into account the concepts of sustainable production (e.g., Cleaner Production, Pollution Prevention, Waste Minimization), which prescribe how to cope with new environmental realities. To sum up, sustainable production within industry must involve innovation and the use of enriched macroalge is a novel application of biotechnology.

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