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MIXOTROPHIC AND HETEROTROPHIC CULTIVATION OF DIFFERENT MICROALGAE SPECIES ON DAIRY BY-PRODUCTS FOR FURTHER SUPPLEMENTATION OF POULTRY DIET

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Abstract: Use of alternative cultivation substrates, for instance dairy industry by-products, can significantly reduce the production costs of microalgal biomass. However, dairy by-products are found to be problematic co-substrate for many microalgae, due to inability to hydrolyse lactose. The focus of this study was to assess the ability of microalgal strains to produce biomass in media supplemented with lactose, glucose and galactose. Among them only microalgae *Chromochloris zofingiensis* was capable to assimilate effectively all three sugars.

Key words: microalgae, dairy industry by-products, *Chromochloris zofingiensis*, β-galactosidase

Introduction

Currently the growing consumption of poultry production, including eggs, requires use of a sustainable nutrient sources for satisfying respectively growing feed demand, and to improve production's quality and animal wellbeing. Recent studies indicate that inclusion of microalgae biomass into laying hen diet can significantly improve the fatty acids profile in eggs and meat, i.e., increasing the content of such polyunsaturated fatty acids (PUFA) as docosahexaenoic acid (DHA) and eicosapentaenoic (EPA). Omega-3 are widely recognized as valuable components of a healthy human diet, as well as can improve laying hen immunity (Saadaoui et al., 2021). For instance, supplementation of hen diet with microalgal biomass, containing only 1% of *Trachydiscus minutus* or 1% of *Japonochytrium marinum* in the feed used, resulted in a significant increase in long-chain PUFA in eggs by 26–66%. Moreover, the addition of 1% *Scenedesmus obliquus* to hen feed resulted in an increased content of carotenoids, i.e., lutein and cantaxanthin, in eggs by 48% and 18%, respectively (Jiru et al., 2021).

Despite of reported improvement in product quality, use of microalgal biomass for poultry diet supplementation remains significantly limited. Currently commercial production of many microalgal species is considered economically unfeasible (Benemann et al., 2018).

In order to facilitate commercial application of certain biotechnologically promising microalgal species, use of different agricultural and food processing by-products, e.g., dairy industry by-products (whey, permeate) can be considered as more cost-effective cultivation media (Chakraborty et al., 2023; Kolesovs & Semjonovs, 2023). Additionally, utilisation of such substrates as whey, can decrease the negative environmental impact caused by dairy industry by-products when they are inappropriately disposed into the environment (Koutinas et al., 2009). Furthermore, studies show that some microalgae species can synthesize biomass and bioactive components (lipids, proteins, vitamins, pigments etc.) mixotrophically and heterotrophically – exceeding their productivity by autotrophic growth (Zhan et al., 2021). Nevertheless, there is still little knowledge on the ability of microalgae to efficiently utilise lactose, which is the main carbon (C) source in dairy industry by-products (Kolesovs & Semjonovs, 2023). It should be taken into account however, that lactose is considered to be a problematic substrate due to inability of many microalgal species to hydrolyse this disaccharide due to lack of β-galactosidase enzyme (Brasil et al., 2017) and a selection of suitable species is required.

This study focuses on screening of microalgal strains obtained from microalgal culture collections or environmental isolates for their ability to grow mixotrophically and heterotrophically on lactose, glucose and galactose as main C sources, in a semisynthetic growth media.

Methods

Selection of cultures

Microalgal strains *Chlorella vulgaris* CCAP 211/111, *Chromochloris zofingiensis* CCAP 211/14, *Haematococcus lacustris* CCAP 34/6 and *Scenedesmus quadricauda* CCAP 276/16 were obtained from the Culture Collection of Algae and Protozoa (CCAP, United Kingdom). Additionally, three microalgae freshwater isolates indicated as M1 M2 and M3 were isolated from local freshwater ponds (Riga, Latvia). Bold's basal medium with triple nitrogen and vitamins (3N-BBM-V) (Yee et al., 2019) was used for cultivation of *C. vulgaris, C. zofingiensis S. quadricauda* and for M1 M2 and M3 isolates. Combined *Euglena gracilis* and Jaworski's medium (EG:JM) was used for *H. lacustris* CCAP 34/6 cultivation (Butler et al., 2018).

Experimental design

In this study microalgae were cultivated in test tubes for 21-day long period. Media supplemented with sugar (glucose, galactose and lactose as C source at 5 g/L) were used in two experimental groups, i.e. mixotrophic and heterotrophic cultivation. Medium without added C source was used as a negative control group for both upper mentioned cultivation types (photoautotrophic cultivation for mixotrophic group). The mixotrophic cultivation has been carried out in a presence of LED light source (3000 lux, day : night cycle – 16:8 h), and heterotrophic cultivation has been carried out in darkness, both performed statically in incubators at 25° C.

Changes in biomass

All experimental groups had four replicates ($n = 4$). After cultivation, samples of cultural liquids were collected, centrifuged at 6000 rpm for 5 minutes, supernatants discarded, and biomass resuspended with distilled water to remove media residues. Afterwards biomass was repeatedly centrifuged and transferred into pre-weighted weighting bottles. Additionally, optical density (OD) of biomass samples was measured at 540 nm in order to estimate growth of microalgae culture.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS (BM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, USA) to compare means at significance level $p = 0.05$.

Results and discussion

This study has demonstrated the ability of certain microalgae to utilize sugars from dairy by-products and convert it into additional biomass by mixotrophic growth. Changes in microalgae biomass synthesis are summarised in Table 1. Obtained results are presented as an initial stage evaluation for further in-depth study of lactose metabolism, biomass and its constituents synthesis.

As shown in Table 1, only *C. zofingiensis* was capable of producing significantly higher amounts of biomass in all types of media supplanted with carbohydrates, including lactose (up to 1.03 ± 0.03 g/L, dry weight) supplemented groups (Figure 1). This obviously can be associated with *C. zofingiensis* ability to produce β-galactosidase, i.e., enzyme required for lactose hydrolysis. Further studies would allow to assess *C. zofingiensis* β-galactosidase enzyme activity, as well as changes in C sources during the cultivation. As shown in Figure 1, biomass production under heterotrophic cultivation conditions was significantly lower (>50%) compared to mixotrophic growth.

Table 1. Changes in microalgal biomass compared to negative control ("+" in case of significant increase) after 21 days of mixotrophic and heterotrophic media supplemented with glucose, galactose, and lactose

Microalgal culture	Cultivation type					
	Mixotrophic			Heterotrophic		
	Glucose	Galactose	Lactose	Glucose	Galactose	Lactose
C. vulgaris	+	+	–	+	+	–
C. zofingiensis	+	+	+	+	+	+
H. lacustris	+	–	–	+	–	–
S. quadricauda	+	–	–	+	–	–
M1	+	–	–	+	–	–
M2	+	+	–	+	+	–
M3	–	–	–	–	–	–

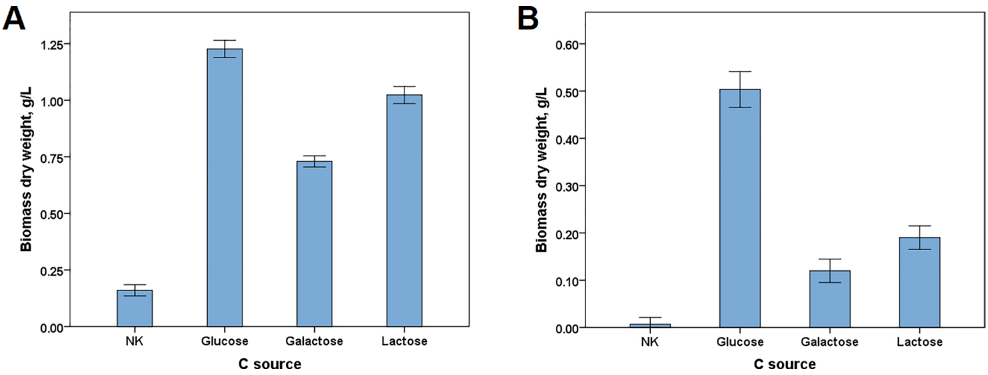

Figure 1. Changes in *C. zofingiensis* CCAP 211/14 biomass synthesis after 21 days of cultivation under mixotrophic (A) and heterotrophic (B) growth conditions with glucose, galactose and lactose as C source or without addition of C (negative control – NK).

Obtained results are in line with data reported previously. For example, it has been shown, that during the evaluation of eight microalgal strains, only three were able to grow on lactose-containing media, and *Dunaliella tertiolecta* (code 117) among them reached highest dry biomass productivity of 0.22 g/L/d on synthetic medium supplemented with lactose as the C source during mixotrophic cultivation. (Zanette et al., 2019). In contrary, another study has demonstrated that addition of lactose can inhibit microalgal growth. Therefore, a selection of suitable strains is necessary in order to achieve higher process feasibility.

Optimisation of *C. zofingiensis* growth conditions, e.g., agitated cultivation, use of more suitable equipment (flasks, bioreactors), pH control and media adjustments (C concentrations, addition of certain growth factors), would allow to achieve significantly higher biomass production rates in further experiments. Additionally, cultivation on lactose-containing substrates, can stimulate the production of valuable bio-active substances such as lipids (including PUFA), pigments (astaxanthin, lutein, β-carotene), proteins, vitamins (Kolesovs & Semjonovs, 2023).

Noteworthy that *C. vulgaris* CCAP 211/111 and M2 isolate showed an enhanced ability to utilize glucose and galactose as main C sources, although, there was no changes in biomass production observed in lactose supplemented media – for both mixotrophic and heterotrophic groups. However, the ability of this strains to utilise glucose and galactose can be considered as beneficial for further formation of microalgal consortia. Use of consortia can stimulate the assimilation of nutrients including high amounts of C present in dairy by-products (Chawla et al., 2020). Further microalgal isolates will be tested using upper mentioned methodology.

Conclusions

Lipid- and pigment-enriched microalgae biomass can potentially be used as a supplement in poultry feed in order to improve both hen immunity as well as production quality. Currently, main limiting factor for wider use of microalgal biomass in agricultural manufacturing still remains its production costs caused by unsatisfying productivity of the process. Therefore, after extensive research and process optimisation efforts, inclusion of *C. zofingiensis biomass* in poultry feeds can be prospective solution to provide such valuable nutrients as PUFA and astaxanthin. Further improvement of microalgae biomass production process, e.g., mixotrophic cultivation on dairy by-products, can significantly improve process`s efficiency and decrease the respective production costs.

Among tested microalgal strains only *C. zofingiensis* CCAP 211/14 was able to produce significant amounts of biomass using all three C sources including lactose. This shows the suitability of *C. zofingiensis* for growth in dairy by-products for biomass production. Further studies will be focused on the assessment of *C. zofingiensis* ability to produce β-galactosidase under mixotrophic and heterotrophic cultivation conditions, as well as changes in biomass composition. Moreover, further evaluation of microalgae biomass effects on poultry health and production quality should be performed to assess the potential of upper mentioned microalgae cultivation`s approach.

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