УДК 573.6.086.83

O. ONYSHCHENKO,
research associate

Dnepropetrovsk State Agrarian and Economic University, Ukraine
E-mail: onish@3g.ua
Dnipropetrovsk, Voroshylova str., 25

INTEGRATED ALAGE CULTURE AS MULTYPURPOSE SOLUTION FOR SMALL PHEASANT FARMS

Обговорюється потенціал культивування мікроводоростей для забезпечення цінною кормовою добавкою мисливських птахів та утилізації відходів виробництва. Вирішується завдання розробки інтегрованої системи культивування з урахуванням ринкових вимог до якості продукції (біомаси), питомої продуктивності та вартості самої системи, яка може забезпечувати й процеси очищення води. Експериментальна робота з інтеграції ланки культивування у виробничий цикл малої птахоферми була виконана для виявлення можливості впровадження у фазанівництві. Доведено, що послід фазанів може бути корисним джерелом поживних речовин для культивування хлорели, яка, крім функції перетворення біогенів, використовується як кормова добавка, що має істотний вплив на продуктивність птиці. Запропонована схема екологічного забезпечення підвищує виробничі показники господарства в цілому.

Ключові слова: *птахівництво, вирощування фазанів, хлорела, біологічно активна добавка, утилізації відходів.*

Algae provide the most promising opportunity to achieve the sustainable production as microalgae aquaculture serves for food supplements, organic chemicals, wastewater treatment, and carbon capture, microalgae culturing technologies are claimed to have vast of economic potential [5].

The idea of integrating of microalgae culturing stage into farming as utilization and supply chain is not quite new. William J. Oswald in the University of California, Berkeley, with his research team had presented the Advanced Integrated Wastewater Pond Systems (AIWPS) technology and results of the first pilot project on integration of the system for chicken farm at the 43rd Annual Conference of Water Pollution Control Federation in Boston in 1970 [4]. Similar projects had been successfully implemented in the Soviet Union almost same time. Although due to farm crisis of 80s in USA and “mass chimization” of agriculture in USSR, the development of the direction had almost stopped completely in both countries [9].

Today, as paradigm had completely changed, according to the principles of “local production to local consumption” and green farming, integration of algae culture as supporting link for animal farm is highly portable and can be established and operated locally, converting waste streams into valuable product [11].

Increasing of production rates in poultry goes mainly in the direction of broiler poultry, mainly by means of the selection, breeding, forage processing and using of chemicals [7]. Same time, the limits of productivity are almost achieved for existing technologies, so there is a strong need in finding of additional reserves to maintaining competitiveness [11]. Despite all positive effects of integration of algae culture has been proved, the need of major investment to introduce the process is the main hindrance on the way of commercialization [3].

For small farm that produce organic or delicacy products, switching to the green non-waste technology, which will provide diversification and improvement of product quality can be easy achievable solution, which primarily does not pose the need of major investment and serious reconstruction [8].

In scope of our research, we studied the possibility of integration of *Chlorella* cultivation system, as part of the ecological maintenance of poultry farm, especially for game birds that are bred for repatriation in nature or producing delicacy products. Such species as pheasant, partridge, virginia partridge are not adapted on the one hand, to completely "artificial" conditions, and on the other hand consumers buy their meat and other products because of their ecological safety first of all [11].

Cultivation of chlorella may be effectively implemented not only as an organic link for waste management [3], and also to provide valuable feed additives for birds instead of synthetic additives, contaminating products and environment [8]. As waste utilization *Chlorella* is very versatile – litter and poultry farms wastewater can be used as a basis for cultivation medias, providing almost complete recycling of liquid wastes [3] on the principles of sustainability and resource conservation.

Effect of enrichment of pheasants diet with *Chlorella* are not reported randomly in the scientific literature, so in our work, we primarily focused on the studying of general applicability of the method and ecological effect for small farm raising pheasants to evaluate general ecological and economic effects.

Materials and methods. The aim of the field experiment was to study the effect of simplified aquatic utilization chain introduction and effects of enrichment of the diet with *Chlorella* suspension for pheasant and determining effective doses of the suspension in the diet.

The experiment was performed in agro holding "Agro-Soyuz" (Dnepropetrovsk, Ukraine), the object of the study was game (hybrid) pheasant production cycle – the farm has parental herd consists of several subspecies of animals and hybrids, randomly farm houses 1500–2000 adult birds which are raised for meet or game. The birds are housed in enclosures designed to accommodate 40–70 birds.

Besides the study of effects of *Chlorella* as additive in the pheasant's diet, the applicability of the technique of producing of *Chlorella* suspension on the farm scale was tested.

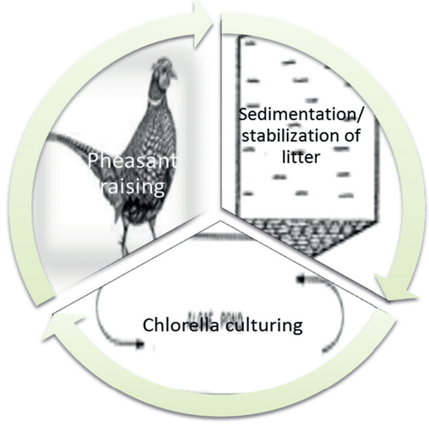
Small settling tank with 100l capacity was made to collect and stabilize litter, to prepare base of organic-mineral media, it was mounted next to enclosures. Litter supernatant from the settling tank was moved to algae pond after suspended parts were settled and bubbles on surface were not observed which happened when media was fully stabilized.

Drinking water was provided to the birds by means of a continuous low rate flow of well located on the territory of the farm, same water source was used for stabilization tank and Chlorella suspension dilution before it was delivered for experimental groups of birds.

Round pond with 100l capacity equipped with fiberglass cover to prevent dirt contamination was installed in open area for algae culture growing. The construction (pond) was provided with bubbling system, which provided gas saturation and mixing in the culture.

Pheasants littler after stabilization was used and the base of culturing media – after soaking litter for 10–30 days as culturing media base for the culturing pond. After inoculation of the pond, the suspension reached the desired concentration (3×10^6 cells per ml) during the week in summer time. After desired concentration was reached, the half of the volume was harvested and fresh portion of culturing media based on litter supernatant was added (pic. 1).

The system approach was used to analyze the performance of the system and investigate the "balance" of inputs, outputs and Δ system changes were performed for total solids, total unoxidized nitrogen and phosphorous [3].



Pic. 1. Simplified utilization/production chain schematic flow pattern

Nitrogen and phosphorous concentration were determined in litter collected from birds, supernatant before culturing and in filtrate from Chlorella suspension after culturing which allowed to count transformation coefficients for nitrogen and phosphorous in the system.

Total Kjeldahl nitrogen was determined in collected litter and *Chlorella* biomass by standard method. Phosphorous was determined after digestion with acid by spectrophotometry method [2].

Concentration of dissolved nitrogen, phosphorous, and total solids were determined in litter supernatant prepared for algae culturing. Determination of ammonia was performed by standard Nessler method. The nitrite determination was performed by standard method with sulfanilic acid and N-1 naphthylethylenediamine, the intensity of the coloring was analyzed spectrophotometrically. Determination of phosphates was performed by standard method of coloring with ascorbic acid combined reagent and measuring for absorbance in a spectrophotometer [2].

Main qualitative parameters were also analyzed for well water which was used for the birds, for settling tank and culturing pond. Nitrogen and phosphorous levels in well water were also considered as a part of nutrients that had been fixed in *Chlorella* biomass [2].

The inoculum of *Chlorella ellipsoidea* for pond seeding with initial cell density 200,000 cell/ml was obtained in laboratory conditions on B3 culturing media. The count of cells was performed using Neubauer Chamber Cell Counting under microscope. Dry cell mass in harvested suspension later was determined as total suspended solids; samples with volume 5–20 ml were filtered through microbial cellulose filters (3 micron pore size) that were dried after at 105 °C for 24 h according to standard methods [5].

Culture growth optimization was performed according to obtained during trial testing microalgae growth curve, quantitative data primary was collected to find out the optimum concentration of *Chlorella* cells and time of culturing between adding media and growth plato, according to the formula of relative growth modified for particular case:

$$k = Log\,(Nt\,/\,No)\,/\,(Tt - To) \cdot 3{,}22,$$

where Nt – cell density at the end of the culturing cycle; No – cell density and the beginning culturing, at $t = 0$; t – time (days); k – relative growth coefficient [3].

Each time *Chlorella* suspension was harvested when growth curve had reached the plato, thus we assumed that culture had some limitation in growth factor and thus nutrients uptake had reached its maximal level. As ultimate nutrient residual nitrate form of nitrogen was determined, as according to fundamental studies *Chlorella* cultures uptake ammonium nitrogen much faster than in other forms and nitrite form of nitrogen is easy reduced.

Obtained data allowed counting the average percent of transformation for nitrogen and phosphorous in this system. The content of nitrogen in *Chlorella ellipsoidea* biomass was determined 6,1 %, phosphorous – 1,1 %, which complies with available data from other authors [3, 5].

Introduction of harvested *Chlorella* suspension into bird's diet was started at the beginning of the egg-laying period. We have formed three experimental groups of birds selected from parental herd. Each experimental group consisted of 35 birds – 29 female and 6 males [4]. Each experimental group was kept in retained in own cage (table 1).

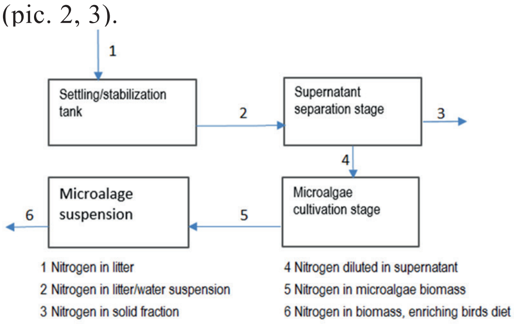
Solid part of the diet and other conditions were the same for all groups of birds and did not differ from those for the other birds in farm.

1. The scheme of the experiment to determine the effective doses of Chlorella for the pheasants

Group	Number of birds	Diet for birds
1 – control	35	OD (ordinary diet) + water
2 – experiment	35	OD + chlorella suspension with concentration (2–3) · 106 cells per ml
3 – experiment	35	OD + chlorella suspension with concentration (1–1,5) · 106 cells per ml

The experiment lasted from April to July, during intense egg laying. Throughout the experiment daily egg production in cages was recorded, egg weight was measured on scales with an accuracy of 0,01 grams, length (for long diameter) and width (at the equatorial diameter) were measured with calipers accurate to 0,1 mm, shape index was evaluated as the ratio of short the long diameter [10]. Vitamin A and carotenoids determination was performed by alkaline saponification, in the presence of antioxidants, followed by liquid extraction with mixture of organic solvents. The absorbance of the obtained carotene extract is finally measured at 450 nm [1].

Results and discussion. According to suggested approach the simplified model of nitrogen and phosphorous transformation in the system were developed to figure out the main transformation stages and develop the basic material – mass balance. The main transformation chains for nitrogen and phosphorous were similar and looked as following (pic. 2, 3).



Pic. 2. Balance and transformation of nitrogen in the system

Nitrogen cycle can be narrowed down to following:

N_l – nitrogen in the litter;

N_d – partially dissolved nitrogen in soaked litter;

N_r – nitrogen in solid litter residue after supernatant removal;

N_b – nitrogen in chlorella biomass.

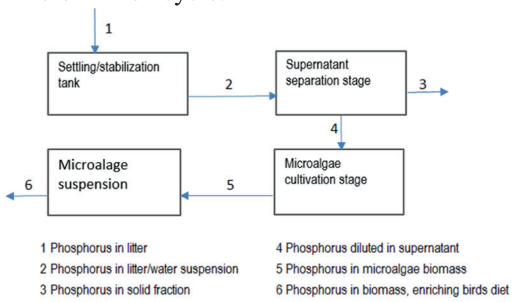
We established two main transformation coefficients in the system:

K_1 – coefficient of nitrogen transformation into soluble form in supernatant;

K_2 – coefficient of nitrogen uptake by microalgae cells in culturing media.

$$N_l = N_d + N_r$$
$$N_d = K_1 \times N_l$$
$$N_b = K_2 \times N_d$$
$$N_b = K_1 \times K_2 \times N_l$$

Phosphorous in the system undergoes same transformation cycle:



Pic. 3. Balance and transformation of phosphorous

Phosphorous fate in the system can be described as following:

P_l – nitrogen in the litter;

P_d – partially dissolved nitrogen in soaked litter;

P_r – nitrogen in solid litter residue after supernatant removal;

P_b – nitrogen in chlorella biomass.

We established two main transformation coefficients in the system:

K_1 – coefficient of nitrogen transformation into soluble form in supernatant;

K_2 – coefficient of phosphorous uptake by microalgae cells in culturing media.

$$P_l = P_d + Pr$$
$$P_d = K_1 \times P_l$$
$$P_b = K_2 \times P_d$$
$$P_b = K_1 \times K_2 \times P_l$$

Thus according to the content of this biogens in the litter, after the K_1 and K_2 empirical meanings are defined, the whole system balance can be projected and scaled up to the certain level.

General qualitative analysis of analysis of water from the well that supplies all farm showed the water quality is meeting the existing farming standards (table 2).

In general, five culturing cycles were performed during experimental period and general nitrogen and phosphorous content in litter, biomass and water for each cycle (table 3).

As it was observed, nutrients transformation process was relatively stable during the whole

2. General hydrocheical characteristics of well water used for farm supply

Parameter	Units	Meaning
Temperature	ºC	10,0
pH	-	7,23
Electroconductivity	µs	1633,0
Total dissolved solids	mg/l	1414,0
Alkalinity	mg.eq/l	2,8
Phosphates	($PO4^{2-}$) mg/l	0,09
Nitrates	(NO_3^{2+}) mg/l	18,0
Ammonium nitrogen	(NH_4^+) mg/l	0,2
Chlorides concentration	Cl^- mg/l	140,0
Hardness	mg.eq/l	10,1

operation. According to low concentration of nitrates in the filtrate from harvested suspension, nitrogen was the factor causing limitation in the culture. Relatively high concentration of residual phosphorous indicates that it is not the limiting factor for the growth process, besides that residual phosphorous concentration was way above permitted levels for water discharge and reuse [2].

We found K_1 coefficient for the nitrogen varies from 0,51 to 0,58 which means that 51–58 % of nitrogen dissolves when we soak litter, K_1 for the phosphorous inorganic compounds were found between 0,73–0,75, which indicates 73–75 % of soluble phosphorous can be extracted by litter soaking.

Coefficients K_2, which basically represent the nitrogen and phosphorous uptake rates by microalgae culture, were quite high and were 0,83–0,95 (83–95 % uptake rate) for nitrogen and 0,72–0,83 (72–83 % uptake rate for phosphorous). In this regard, we assume there is a need in the balance correction, which may be achieved by adding nitrate fertilizer in the certain proportion to the supernatant, in this case both biogens might be more fully utilized by microalgae culture.

During the period of the experiment, harvested Chlorella suspension was used instead of water for drinking in two experimental groups. We observed no direct correlation between the concentration of chlorella in the diet and egg productivity, so egg productivity in the second group was higher than in the control group and the third group was lower than in control (table 4).

As much as high variation indicates that the impact of individual factors on group performance. On the influence of individual fac-

3. Concentration of nitrogen and phosphorous in the medias in the modeled transformation chain

Sampling date	Wet content of litter, %	Total nitrogen concentration in litter, g/kg	Total phosphorous concentration in litter, g/kg	Total weight of litter collected for stabilization, kg	Chlorella biomass concentration, g/l	Residual nitrogen in water after harvesting of Chlorella suspension, mg/l	Residual phosphorous in water after harvesting of Chlorella suspension, mg/l
15 April	67,5	24,6	8,31	0,35	0,63	8,30	47,87
03 May	67,5	24,58	8,32	0,37	0,7	8,80	36,33
04 June	67,4	24,61	8,42	0,36	0,65	8,70	48,56
25 June	67,2	24,59	8,43	0,34	0,67	9,20	58,80
17 July	67,4	24,60	8,44	0,34	0,67	7,60	61,21
Average	67,40	24,60	8,38	0,035	0,66	5,70	50,55
St. dev.	0,12	0,01	0,06			0,60	9,93

4. Egg productivity in experimental groups

Group	Meaning	April	May	June	July	
1	Minimal egg productivity per day	7,0	9,0	3,0	5,0	
	Maximal egg productivity per day	17,0	17,0	19,0	14,0	
	Average egg productivity per day	12,9	13,0	10,2	9,0	
	(Cv)	2,8	2,2	3,8	4,6	
	General amount of eggs					822
2	Minimal egg productivity per day	5,0	8,0	4,0	6,0	
	Maximal egg productivity per day	20,0	20,0	17,0	9,0	
	Average egg productivity per day	14,4	13,6	11,2	7,7	
	(Cv)	3,7	2,6	3,4	1,5	
	General amount of eggs					881
3	Minimal egg productivity per day	8,0	2,0	2,0	1,0	
	Maximal egg productivity per day	16,0	16,0	19,0	11,0	
	Average egg productivity per day	13,6	10,5	9,7	7,7	
	(Cv)	2,5	3,4	4,4	5,8	
	General amount of eggs					743

tors indicated by morphometric parameters eggs collected during the experiment. It is known, that the color and shape index of eggs depends on the subspecies and age of laying hens [6, 10], so as we analyzed these figures, as revealing the background of differences in experimental conditions between groups. In the first and second group had average dark eggs share 71,5 and 72 respectively, while the third – 54 %.

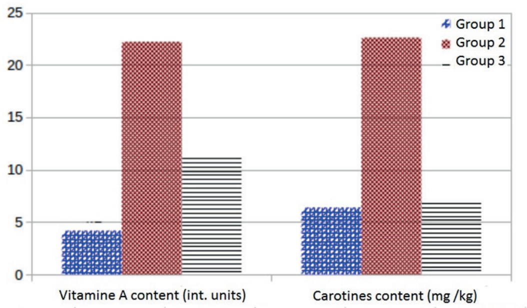
5. Egg qualitative characteristics in experimental groups

Group	Meaning	Egg mass	Index of shape
1	Minimal	26,66	1,23
	Maximal	36,18	1,34
	Average	30,54	1,27
	(Cv)	2,77	0,03
2	Minimal	29,94	1,22
	Maximal	33,69	1,31
	Average	31,85	1,27
	(Cv)	1,35	0,03
3	Minimal	28,37	1,22
	Maximal	34,04	1,4
	Average	31,53	1,29
	(Cv)	1,91	0,06

Average index form in the first and second group and was 1,27 and 1,29 in the third. There was noticeable increase in average egg weight to 4,4 % ($P<0{,}001$) in the second group receiving the high dose of chlorella and by 3,3 % ($P<0{,}001$) in the third group receiving low-dose versus control (table 5).

Significant differences in the content of carotenoids in egg indicate that *Chlorella* suspension was properly digested by birds, and high doses are more effective and therefore more appropriate to use.

Significantly different content of carotenoids and vitamin A in eggs of birds receiving Chlorella suspension was observed – carotenoids content had increased in 3,5 and 1,2 times ($P<0{,}05$) in the second and third groups, respectively, and vitamin A in 5,3 and 2,7 times ($P<0{,}05$) compared with the control (pic. 4).



Pic. 4. Content of carotenoids and vitamin A in eggs experimental groups

It should be noted that vitamin A is not reduced during the breeding period in the second and third group, which, according to literature data, is not typical for laying birds [10].

As we observed noticeable effect of dose 2–3×106 cells per ml of Chlorella in suspension for laying birds it was decided to introduce suspension in the same concentration for hatching chicks during first two weeks while they are housed in separate rooms with temperature control. Control and experimental groups of chicks were housed in different rooms and control group had just water for drinking while chicks in the experimental group got Chlorella suspension. The average weight gain in within first two weeks after hatching in the experimental group was 23,5 % ($P < 0{,}001$) higher than in the control group.

Conclusions

Like any other biological supplement Chlorella suspension shows no immediate effect, but significant changes observed in the experiment indicate the feasibility of more detailed studies and long-term ex-situ.

Litter converting into biomass according to proposed transformation chain can be performed with high efficiency during spring – summer period, which can provide closed cycle of local production and local consumption of the supplements (Chlorella) to significantly improve products (egg) quality and general hatching rates for the farm.

Introduction suspension of Chlorella in the diet of pheasants in the breeding period is fairly simple from a technical point of view by enriching the diet of pheasant, in the absence of risk of the overdose and toxicity that is unavoidable in case of using of synthetic additives.

References

1. ДСТУ 4687:2006. Комбікорми, премікси, вітамінні препарати, продукція птахівництва. методи визначення вітаміну А, Е, В2 та каротиноїдів.

2. Санітарно-гігієнічні вимоги до води та водопостачання сільськогосподарських підприємств. Навчальний посібник / [Укладачі: *М.О. Захаренко, В.М. Поляковський, Л.В. Шевченко* та ін.]. – Вінниця: Видавничий центр ВНАУ, 2012. – 244 с.

3. *Bennemann J.* Systems and Economic Analysis of Microalgal Ponds for Conversion of CO2 to Biomass / *J. Bennemann, W.J. Oswald.* – Department of Energy, 1996. – 235 p.

4. *Cetin O.* Breeding Possibilities of Pheasants (P. Colchicus) In Intensive Conditions and Cold Climatic Zone: II Growth and Carcass Characteristics / *O. Cetin, K. Kirikci, C. Tepeli* // Veteriner Bil Derg. – 1997. – V. 13, № 1. – P. 69–76.

5. *Chae S.R.* Single cell protein production of Chlorella and carbon dioxide fixation in an innovative photo-bioreactor / *S.R. Chae, E.J. Hwang, H.S. Shin* // Bioresourource Technology. – 2006 – № 97(2). – P. 322–329.

6. *Esen F.* The effect of egg production, hatchability and egg characteristics in pfeasents / *F. Esen, O. Osbey, F. Gen Ç.* // Jornal of animal and veterenary advances. – 2010. – Vol. 9, № 8. – P. 1231–1237.

7. Guidance for the preparation of dossiers for technological additives EFSA: Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) // EFSA Journal. – 2012. – № 10(1). – 23 p.

8. International Federation of Organic Agreculture Movements (IFOAM) Acess: www.ifoam.org

9. *Purvina S.* Insight into the studies of the practical use of microalgae in the former Soviet Union / *S. Purvina, M. Balode* // Phytochemistry Reviews. – 2004. – Vol. 3, Issue 3. – P. 423–438.

10. *Thomas V.G.* Influence of date off egg production and diet on pheasant chick development / *V.G. Thomas, E.D. Bailey* // Canadian journal of zoology. – 1973. – Vol. 51. – P. 1149–1154.

11. *Васильєва О.О.* Історія та перспективи розвитку фазанівництва в галузі птахівництва України / *О.О. Васильєва* // Вісн. Полтав. держ. аграр. акад. – 2009. – № 2. – С. 58–62.

Reviewer – doctor of agricultural sciences, professor ***N.N. Charitonov***