SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING, AGRONOMY AND VETERINARY MEDICINE

Polish - Ukrainian Cooperation

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

THE UKRAINIAN AGRICULTURAL GROUPS STATE AND AGRO-MACHINERY ROVISION

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KEYWORDS

agricultural machinery, technical provision, agricultural production, technical equipment, intensive technologies, tillage, soil compaction, vehicles, machinery efficiency enhancement

ABSTRACT

The study is intended to examine the reproduction and efficient application of technical potential of the agricultural production. Analysis focuses on the activities of agricultural enterprises, and the influence of the machinery availability, its amount and quality on the economy results. The presented findings reveal the connection link existing between agricultural commodity producers and agro-machinery manufacturers, define technical and economic premises of establishment and development of the corporate technical servicing. The major strategies of the development of sustainable provision and efficient machinery application under the conditions of innovative technologies application, are circumscribed.

On the condition of provision of the agricultural production with the latest agricultural machinery and advanced technologies depend its further development, population welfare, working position and sustainable income security of the rural areas and their inhabitants. Therefore, the underlying problem, being quite relevant and profound, requires careful consideration and can be further developed in the scientific field.

As the result of detailed study, the explicit development plan of the provision of the agricultural enterprises with physical infrastructure, the development strategies of the rural areas, and specific business plans can be elaborated.

INTRODUCTION

The essential marketing levers of the equivalent exchange between the national economy sector and the agro-industrial complex (AIC), weren't defined under the entire period of economic reforms in this country, which predetermined, for its part, the unfavourable socio-economic and marketing environment of the agrarian sector, under the conditions of which, together with the price disparities of agricultural and industrial products, the husbandry has been functioning till nowadays. This is happening despite the fact that the legislative acts, a number of decrees of the President of Ukraine, and the resolutions of the government stipulate the equivalence of exchange as an obligatory condition for the free development of the agricultural enterprises economy.

Such circumstances created the marketing environment of the AIC, in which the agricultural producer with low income can't renew the equipment, being 80-90 % obsolete nowadays, and the agro-machinery manufacturer can't realize the equipment being produced,

and, consequently, cuts down the manufacturing output, destroying, at the same time, the industry of tractor and agro-machinery engineering.

Such a state of facts requires the fundamental analysis of the system of technical provision of agricultural producers and machinery manufacturers supply of machine-building enterprises. The factors and impulses, creating unsatisfactory condition in these areas are to be revealed. The study is also to develop and prove measures, and to set forth the ideas, considering this problem resolution.

MATERIALS AND METHODS

A number of domestic scholars' studies were dedicated to the definition of the place and the purpose of the technical provision of the AIC agrarian sector. Among them I. K. Bilousko, V. H. Bilskyi, L. M. Budniak, A. V. Burylko, M. I. Herun, M. I. Hrytsyshyn, P. A. Denysenko, T. S. Ivashkiv, A. P. Korzh, I. P. Masl, M. M. Mohylova, S. V. Petryk, V. O. Pytulko, H. M. Pidlisetskyi, L. V. Pohorilyi, V. L. Tovstopiat, V. M. Trehobchuk, V. P. Yakovenko. Among foreign scholars in this area H. Donaldson, E. Kain, O.Kantor, A. Koller also should be mentioned.

At the same time, despite the attention of science to the problems of technical provision of the agricultural sector, the actual state of affairs in this industry is still far from desirable in Ukraine. In particular, a lot of scientific developments, despite their theoretical correctness, cannot be applied in practice due to their inconsistency with the actual situation in the industry. Technologies and techniques of such a work are poor and imperfect. There are problems even at the level of the conceptual apparatus which urges the determination of the cause of an existing unsatisfactory condition, already at the stage of the development of methodological bases of the problem. Thus, conducting research towards improvement of technologies and techniques and methodological bases of development of the technical provision systemof the AIC agrarian sector is quite relevant, which determined the subject of this study.

The following scientific methods shed light on the issue under study: historical, statistical, methods of comparison and analysis.

RESULTS

There is still a necessity of modern machinery being developed and producedin Ukraine, and it depends on the two main circumstances. At first, in the beginning of the nineties about the third part of all the technical equipment required by the agrarian sector of Ukraine was produced in the country. In the second place, the certain constitutional changes took place over the period of independency. In particular, the production of the

traditionally unprofitable products, requiring the expenditure of much labour – vegetables, potatoes, milk and beef – moved from large agricultural enterprises in the private farms. They require compact equipment, the respective manufacturing of which isn't organized yet. The current situation is peculiar due to that fact that innovative part is tightly interlaced with an ordinary supply of materials and machinery. However, the innovative role of technical equipment in the mechanisms of economic relations still can be identified in case of the purchase or the agro-technical servicing and also while analysing the efficiency of technology application.

The infrastructure-creating role of technical equipment is generated by the impartial necessity of arranging certain conditions for proper machinery application in the production sphere. As is generally known, the infrastructure (transport, connection, routes, etc.) is a totality of areas providing stable economy functioning. Likewise, there are a number of measures that are to be taken to ensure the efficient mechanisation means application at the agricultural enterprise. Of great importance is a personnel training. The personnel of mass professions –tractor-operators, and higher qualification – engineers and technicians are required. As far as, the following category of workers is enrolled into economy on an on going basis, the overwhelming majority of enterprises defray the expenses of their training at the corresponding educational establishments. The number of the machinery constantly increases, and the personnel-training costs have become the constant component of the enterprise outgoings. The dealer network also can be considered to be personnel assistance in technique. The personnel is not the only component of the agricultural enterprise service scheme being required for the efficient machinery application, the other one includes the following material and technical premises : establishing accommodations, or at least specially equipped areas for its maintenance, diagnostic centres and repair shops with the appropriate equipment, spare parts storehouses, and storage facilities for fuel and lubricants keeping and units fuelling, vehicles for fuel and spare parts delivery, and so on.This is a kind of service infrastructure that also has working positions of repairmen, security guards, vehicle drivers, etc. To accommodate the production facilities the land is needed and the facilities must meet certain technical, sanitary, and social requirements. A certain complex of means of production and workers that is to be rationally arranged is gradually being formed.

The technical equipment part is undoubtedly quit important and becomes apparent mainly through forming of economic results of the agrarian manufacturers affairs. At first, technical equipment ensure the receipt of the plant growing gross output, its post harvest handling, dispatch for storage, and in some cases – even the transportation to the place

of processing. Taking into account that fact that the technical equipment performs the most power-intensive operation of the plant growing production, the assertion that mechanization means have completely covered the whole technological cycle of the plant growing production, especially the one concerning large agricultural enterprises, can be made. The machinery also influences the process costs and the plant growing production cost. Suffice it to say that sum total of all the Ukrainian agricultural enterprises plant growing costs on petroleum products, electrical energy, spare parts and repairing materials account for about 40% of process costs sum total.The under study technologies of growing winter wheat differs in degree of intensity and yielding capacity, respectively – extensive 2-3 t/ha, conventional 3-4 t/ha, intensive 5-6 t/ha, and high 8-10 t/ha –show that in the extensive and conventional technologies (Appendix A) the major share in the total structure of expenses is the cost of tractor-implement unit (TIU) – 69.2 and 90.6% respectively. Therefore, the main reserve of profitability increase of these technologies is the reduction of costs for the TIU and fuel.

Under the intensive and high technologies the costs of process materials (fertilizers, means of plant and seed protection) run high with the increase of planed yielding capacity compared to TIU costs. Thus, process materials costs of the yield capacity amounting to 5 t/ha dominate TIU and fuel costs 1.3 times, while the yield capacity amounting to 10 t/ha –3.1 times.

Thus, cutting down the process materials expenses, by means of improvement their application efficiency – precise agriculture, dosing fertilizers according to the stages of organogenesis, and so on – becomes the main reserve of the economic efficiency rise.

As a result of depiction of expenses scale dependency on the yield capacity, compared to price, the dependency on certain technology can be observed. As the Appendix A shows, extensive technology with the yield capacity amounting to 2t/hais unprofitable (-35.3%), because of heavy TIU expenses and low price on poor-quality corn. Though, the substitution of conventional tillage and sowing for the direct sowing under this technology cuts down the TIU expenses, but on the whole, it doesn't result in such a technology becoming a profitable one. The extensive and conventional technologies with the yield capacity amounting to 3 t/ha are characterized with the minorunprofitableness – 2.5%.

The efficiency of the technologies can be enhanced by means of:

- substitution of ploughing for shallow and medium tillage with multifunctional soil-cultivating units;
- substitution of conventional drill sowing for direct band sowing with the combined units;

- preference for direct combine-harvesting over separate collection, under favourable conditions of the grain crops harvesting;
- introduction of technological method of plants foliar feeding with urea solution to enhance the quality of the grain.

Conventional technology with the yield capacity amounting to 4 t/ha, and intensive with 5 t/ha respectively, under the zero tillage (no-till) and direct sowing conditions may both become the most efficient. This can be explained with the fact of their application of particular technological operations, combining pre-sowing tillage and band sowing, which, compared to conventional tillage and sowing, ensure cut down of TIU expenses, moisture keeping, contribute to uniformity of the plant nutrition area, and increase the yield capacity in the whole. The use of crushed plant residues and small doses of fertilizer ensures the effective maintenance of the natural fertility of the soil.

Intensive technologies with the yield capacity amounting to over 6 t/ha, and high technologies – 8-10 t/ha respectively, despite of the considerable total expenses, can be efficient, in case of guaranty that yield capacity amount is the same as it was planed, and high quality of the grain. Therefore, planning yield capacity amounting to over 8 t/hawith the predicted profitableness is only possible in a friendly to winter wheat cultivation soil-climate zones.

Technical equipment influences over the production output and the expenses of livestock sector through plant-growing production – the fodder, which is the key resource of the livestock. In the agricultural enterprises of Ukraine fodder accounts for over 70% of the pecuniary and about 30% of total process costs of livestock. Therefore, the contribution of technical equipment in the formation of the economic results of agricultural production can be considered as a decisive one.

The above mentioned can be confirmed with a data on the structure of process costs of agricultural enterprises. The share of the technical equipment related costs (petroleum products, electrical power, spare parts and repair materials, amortisation) in total sum of pecuniary process costs of Ukrainian agricultural enterprises accounts for about 30% and has been stable in recent years.

Environmental consequences of the technical equipment application in agriculture are better known, rather on qualitative than quantitative side, as negative. Basically they are evident in soil compaction, which may even extend to the full depth of the soil profile. In modern tillage technologies, the area of traces of tractors and machines accounts for 90% of the area of the field. A strong compaction can cause irreversible changes, namely

degradation of soil structure, as far as the depth of compaction exceeds the depth of the topsoil. The destruction of soil-covering can't be quantitatively measured in Ukraine, because this is a disaster. However, the negative effect of compaction is neutralized with the accumulation of stubble and root mass, the moist soil freezing, and also due to special systems of cultivation (minimum tillage).Thus, negative environmental consequences of the technical equipment application in agriculture aren't inevitable.

However, it should be taken into account that the minimum tillage put forward entirely new demands for the livestock technical equipment.

First of all, it should be mentioned, that the negative consequences of the total domination of ploughshare, as the main instrument of the fields tillage, are notorious sincethe30-s – 40-sof the last century. World-wide known among the farmers E. Faulkner suggested tillage system under which the disks were used instead of plough. However, at the time, and in the next few decades there were not the main prerequisites of the plough less tillage switchover – highly effective herbicides. Gradually this problem was solved and, due to the wide use of chemical weeding in the industrial practice, it was possible to completely or partially abandon the mechanical tillage of the field, which threatened with erosion, structure deterioration, and reduced fertility. Modern tillage technologies can vary from the conventional ploughing to zero tillage (sowing in uncultivated soil).Methods, occupying an intermediate position, are called minimum tillage, that is, one that provides minimal mechanical effect on the soil-covering of the arable. It is believed that the minimum and zero tillage saves fuel and labour, it increases productivity, reduces soil compaction by tractors and agricultural machines.

Among numerous methods and techniques of minimum and zero tillage, the best four were selected: autumn chiselling (sub soiling), band sowing, regular ridge sowing, direct sowing in untilled soil. All of these methods simultaneously perform soil protection functions. Chiselling is performed with the help of previously-known machinery, and it slightly differs from conventional tillage in terms of erosion prevention. The new machinery was created for band sowing. The Kinse Mfq (USA) combined machine may serve as an example. It has a set of disks with a diameter of 45 cm, which pulverize nutrient residues and loosen the topsoil only within the band limits. Behind the disks the cultivator teeth are mounted, then – rotary tools for incorporation of herbicides in the soil, rod-shaped drums and the sowing coulters.

The production of machines for sowing in ridges in the United States began in the sixties of the last century, and in the mid-eighties the firm Fleisher and Hiniker

Manufacturing began to produce a full set of machines for this method. The stubble drillers for the direct sowing were created on the basis of the chiselling coulter [1, 2, 13, 4, 5].

The provision of agricultural production with modern advanced technical equipment at an optimal level, high efficiency of its application, timely updates, development of advanced technologies of agricultural production, reasonable forms of organization of repair and technical servicing and supply, availability of qualified technical and engineering personnel, is the guarantee of high- skilled, in fixed agro-technical terms, accomplishment of all the technological processes and operations and optimal cost of labour and means of obtaining agricultural products.

Ukraine has reached the highest level of the provision of agricultural production with technical equipment in the late eighties and early nineties. Thus, there were 511.5 thsd tractors, 103.4 thsdgrain harvesters (105.2thsd by the end of 1991), 15.3 thsd corn harvesters, 20.1thsd beet harvesters, 75.4 thsdmilking installations and units in the agricultural enterprises at the end of 1992. By that time, the provision of agricultural production with technical equipment, by different types of machinery, was approximating to technological demand [6,7].

In subsequent years, because of ill-conceived government policy concerning agriculture, the instability or absence of legislative support, the inconsistency and indecision of the executive bodies in the agricultural reform, the premature liberalization of prices of material and technical resources and mistaken pricing policy of state authorities concerning the products of agriculture, attempts of the state to solve financial and economic problems of national economic complex at the expense of the village, as it was in Soviet times, resulted in a sharp violation of the equivalence of inter-industry trade and the solvency decrease of rural producers. This caused a slowdown in the updating of technical facilities and, as a consequence, of the overall level of technical equipping of agricultural production with tractors, combines and other means of mechanization of technological processes and operations.

The lowering of the technical equipping level of the agricultural production started in the early nineties of the last century and continues till nowadays. Completely obsolete machinery is putting out of commission, and the agricultural producers have no money to replace it with the new one. The equipment of agricultural production with machinery has reached a critical point, which is fooled with the complete de-industrialization of agricultural production and the cessation of commercial production of different types of agricultural products. At present, the provision of the agricultural enterprises with the agro-machinery

accounts only for 30-60% of its technological demand. It is also should be mentioned, that the technical equipment that is used by peasants is 85-90% obsolete. It has already exceeded its depreciation period, and a great part of it is usually operated during 2-3 such terms. Because of the high obsolescence and lack of funds to agricultural producers for the full repair and service work each year from 25% to 40% of the available machinery do not participate in production. All this leads to a reduction in acreage, and in case of non-fulfilment or partial fulfilment, together with the violation of the agro-technical terms of conducting of technological processes and works, results in decreasing of agricultural cereals yielding capacity, and significant losses of already grown products because of harvesting deadlines prolongation. Annually, because of agricultural production being technically underequipped, Ukraine loses about a third of gross yield of agricultural products, representing in money value 15-18 billion UAH, and due to lack of grain-harvesting machinery, and consequently a significant increase in terms of cereals harvesting, the grain is lost in the amount of about 8-10 billion UAH [8].

Due to the reduction of agricultural producers solvency, the volume of production of agricultural machinery in the domestic machine-building factories is tenfold reduced, the agro-technical servicing companies are on the verge of bankruptcy.

The provision of Ukrainian agrarian sector of economy with machinery should be considered from the standpoint of functional sufficiency. The number and quality of technical equipment should be sufficient to perform its various functions, which were discussed earlier. Accordingly, the characteristic of the state of provision may be, firstly, a symptomatic one, and secondly, adirect one. Symptoms of poor provision, namely the variety of displays of insufficient mechanization means accomplishment of their tasks, are the following: delayed fulfilment of complex of agricultural works, their high labour intensity, and even unsatisfactory results of managing that find expression in unstable production volumes and sales, its low profitability or even losses, high cost, etc.

The direct characteristic of the condition or level of provision –is a variety of comparisons: availability of machines with technologically justified demand for technical equipment, availability of machines and mechanisms on a certain date with their actual number in previous years, the availability of mechanization means in Ukraine with the similar indicators in other countries etc. Of course, the symptomatic characteristic is less convincing than the direct one, as far as, between the machines availability and the economy results exists the factor of culture and level of their use. And they can be low even for such a reason as the low professional level or just an insufficient number of qualified mechanization personnel,

poor repair and diagnostic facilities, the irregular provision with fuel and spare parts and because of many other reasons.

Therefore, in the evaluating of the provision state of the Ukrainian agricultural sector of economy with technical equipment, the preference is shown to direct indicators based on the state statistical reporting data, in particular, to such statistical bulletins as " The Annual Availability and Traffic of Tractors and Agro-Machinery in Agriculture", "The Availability of Agro-Machinery and Power Capacities in Agriculture" [6,7,9,10,11,12,13].

Consequently, the number of harvesting machines reaches the critical point at which, if the trend of permanent reduction remains in the future, there will be nothing left to harvest the agrarian production with.

Already, due to a sharp harvesters decrease in the number, the cereals harvesting periods are prolonged up to 30 days or longer, although the optimum period lasts for 10-12 days. This prolongation of harvesting terms causes significant losses of grain being grown (Fig.1.). If on the 5th day after the full ripening of the grain, the losses of grain from its shedding reach 3 – 4%, than on the tenth day they increase up to 11%, and with prolongation of the harvesting period up to 30 days, they reach 30-33% and continue to increase during the following days [14].

On average, if, by the harvest storage, about 7% of the cultivated grain is lost in 10 days, and about 15% – in 20 days, than, under the prolongation of the harvesting period up to 30 days, average losses accounts for21% of the cultivated crop.

The technology of agricultural production stipulates the execution of a great number of technological operations and processes during the period being optimal, in terms of agro-technical, climatic and weather conditions. The most advanced technology is only effective when all its parameters are unrestrictedly fulfilled. First and foremost, this is peculiarities of agro-technical methods application, their time constraints, and timely transition from the preceding stage of work to the following one.

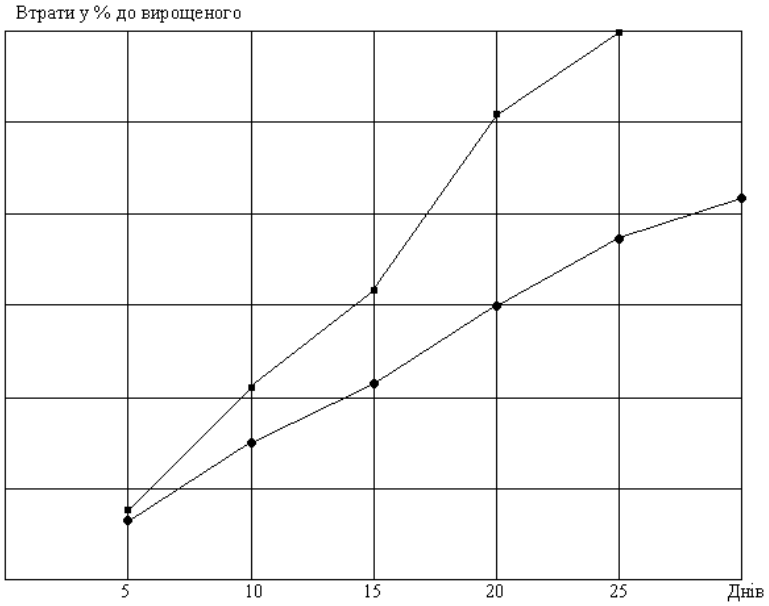

FIGURE1.Percentage losses of grain from its shedding compared tithe percentage of the cultivated one, depending on the harvesting period from the moment of ripening [14].

■——■——grain losses on a certain day after the ripening;

●——●——grain losses, in a certain harvesting period, percentage per cultivated one.

Violation of these requirements may reduce to zero the benefits of even the most advanced technology. To satisfy these requirements is possible only with the necessary technical means in an amount that provides all the technological requirements. These requirements can only be satisfied if having required machinery in quantity being enough to meet the technological demand.

Under the current conditions of considerable deficiency of agricultural machinery, time constraints of the technological operations execution are often violated, and some of them are not executed at all. Even the violation of the time constraints of the commencement of the technological operations execution leads to a significant shortage of products (Table 1) [15].

The data, presented in the Table 1, considering the relative daily-average agricultural yield capacity losses as percentage of final results, in case of the deviation from the optimal commencement term of the basic technological operations in all the natural economic zones of Ukraine, shows that the execution of fieldwork, especially the optimal terms of their beginning are not bound to specific calendar dates or periods of time, but, to a large extent, dependent on climate and weather conditions and environmental phases of growth and development of plants.

Table 1. The relative daily-average losses of the agricultural yield capacity, considering the deviation from the optimal commencement term of technological operations (percentage) [14]

Crops	Zone	Technological operation					
		Ploughing	Pre-sowing cultivation	Sowing	Mowing in swath	Swath threshing	Direct combine harvesting
Winter Wheat	Polissia	1.30	0.70	0.90	1.65	1.55	1.70
	Forest-	0.90	0.60	0.80	1.70	1.50	1.90
	Steppe	1.00	0.40	0.90	1.80	1.60	2.00
Winter Rye	Polissia	1.10	0.55	0.80	1.60	1.70	1.70
	Forest-	0.80	0.55	0.70	1.81	1.60	1.90
	Steppe	1.00	0.55	0.80	1.90	1.70	2.00
Winter Barley	Polissia	1.20	0.56	0,82	1.70	1.65	1.80
	Forest-	0.80	0.56	0.75	1.73	1.52	1.95
	Steppe	0.90	0.56	0.83	1.85	1.65	2.10
Spring Wheat	Polissia	0.22	0.28	1.10	1.58	1.52	1.60
	Forest-	0.20	0.30	1.20	1.70	1.60	1.73
	Steppe	0.24	0.34	1.40	1.73	1.65	1.76
Spring Barley	Polissia	0.23	0.28	1.12	1.63	1.54	1.65
	Forest-	0.20	0.32	1.20	1.74	1.60	1.78
	Steppe	0.21	0.34	1.42	1.78	1.67	1.80
Oats	Polissia	0.23	0.28	1.18	1.63	1.50	1.67
	Forest-	0.18	0.32	1.22	1.67	1.53	1.69
	Steppe	0.21	0.34	1.26	1.69	1.59	1.71
Millet	Polissia	0.08	0.30	1.24	1.54	1.23	X
	Forest-	0.12	0.35	1.20	1.60	1.30	X
	Steppe	0.10	0.37	1.14	1.67	1.35	X
Buckwheat	Polissia	0.25	0.32	1,23	1.60	1.54	X
	Forest-	0.20	0.34	1.21	1.62	1.58	X
	Steppe	0.21	0.36	1.29	1.67	1.63	X
Pea	Polissia	0.22	0.28	1.16	1.40	2.30	X
	Forest-	0.21	0.33	1.18	1.45	2.40	X
	Steppe	0.20	0.34	1.22	1.49	2.57	X
Vetch for seeds	Polissia	0.22	0.27	1.15	1.39	2.29	X
	Forest-	0.21	0.32	1.17	1.44	2.39	X
	Steppe	020	0.33	1.21	1.48	2.56	X
Maize for corn	Polissia	0.08	0.40	1.60	X	X	1.80
	Forest-	0.07	0.32	1.29	X	X	1.60
	Steppe	0.01	0.25	1.10	X	X	1.30

Winter rape	Polissia	0.90	0.70	1.00	1.40	1.27	1.46
	Forest-	0.70	0.60	0.80	1.50	1.22	1.57
	Steppe	0.80	0.40	0.90	1.55	1.35	1.64
Spring rape	Polissia	0.25	0.18	1.09	X	X	1.60
	Forest-	0.20	0.20	1.13	X	X	1.98
	Steppe	0.23	0.22	1.19	X	X	2.21
Soya	Polissia	0.24	0.20	1.22	X	X	1.51
	Forest-	0.23	0.21	1.25	X	X	1.93
	Steppe	0.22	0.22	1.29	X	X	2.17
Sunflower	Polissia	0.26	0.37	1.50	X	X	1.80
	Forest-	0.22	0.35	1.36	X	X	1.90
	Steppe	0.22	0.30	1.10	X	X	2.00
Sugar beets	Polissia	0.30	0.38	0.86	X	X	0.25
	Forest-	0.25	0.36	1.14	X	X	0.25
	Steppe	0.25	0.35	0.56	X	X	0.35
Annual grasses forhay	Polissia	0.40	0.38	1.40	X	X	2.30
	Forest-	0.35	0.33	1.20	X	X	3.00
	Steppe	0.30	0.33	1.40	**X**		3.60

Taking into account the current state of technical equipment of agricultural production, while the technological demand for basic agro-machinery being 40-60 % met, it's almost impossible to avoid these losses. Thus, if the effective measures to provide the agricultural producers with technical equipment at the level of technological demand are not taken in the nearest future, then the question is not only in the enhancement of the efficiency of the agrarian sector of Ukrainian economy, but the point is whether it's possible to preserve the domestic commercial crop-farming at all.

CONCLUSION

Thus, according to the findings of the conducted research, it can be clearly argued that the provision of Ukrainian agriculture with agro-machinery is quite low and its condition is pretty neglected. This leads to significant losses during the agricultural production harvesting, violation of the crops cultivation technology, significant soil compaction, and deterioration of the ecological state of production in the agriculture.

Therefore, with a purpose of strengthening the agriculture physical infrastructure, the explicit action plan, which considers its substantial enhancement, should be developed. This takes its beginning with the agricultural groups proprietors providing of state incentives for their supply of machinery: state grant programs, rural areas development programs, grants

for agricultural producers, and concluding with the leasing, credit, financial, and investment incentives.

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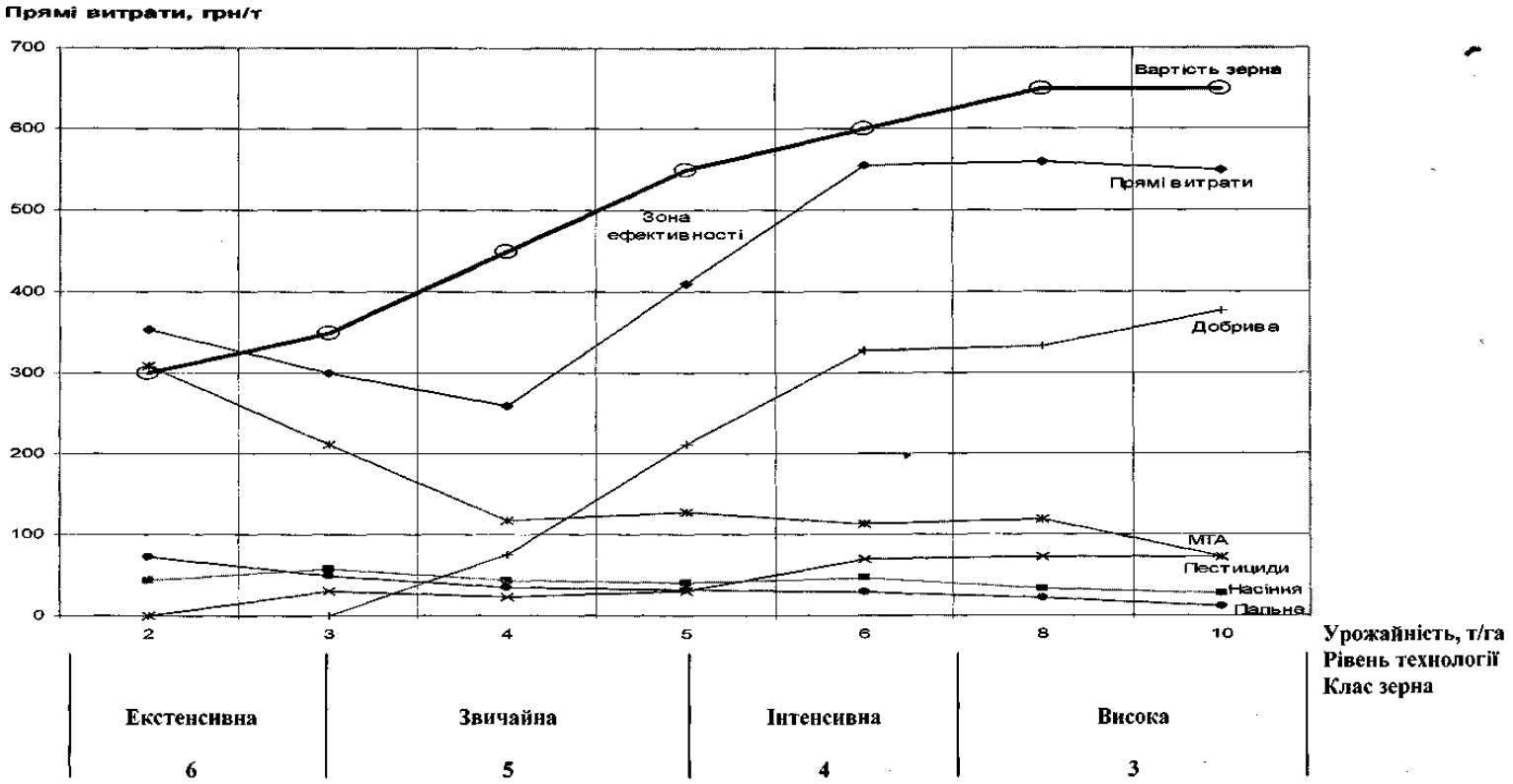
APPENDICES

Appendix A

The winter wheat costs and efficiency dependence on the yield capacity and quality level



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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

ECONOMIC SECURITY AND RISK MANAGEMENT IN THE AGRICULTURALSECTOR

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KEYWORDS

risk management, hedging, agricultural production, economic security, state control

ABSTRACT

The paper reports on research into economic security and risk management in agriculture. A number of themes are concerned including the main types of risks and threats that prevent the effective enterprise activities in terms of gross output formation and export return in grain branch of national economy. The article aims to highlight the strategic guidelines, components of economic security mechanism and risk assessment. The aims of development, indicators of threats, correlation schemes of profit and risk are systematized and summarized to make motivated and rational management decisions and to find the ways for risk prevention. The study demonstrated the need for system of state control of agricultural support with the help of the mechanisms of institution improvement and programs of balanced development of the agrarian sector and the use of economic security tools as an effective risk management instrument.

INTRODUCTION

The development of the world economy is accompanied by gradual changes in the directions of realization the relationship between their participants. We deal with the growing competition, increasing demands on highly-qualified staff of the subjects of market relations, including the criteria of business environment, ecological compatibility and security of forming and using the resource component. These trends of globalization processes are discovered in the agricultural sector, and became the first and foremost direction of economic growth in Ukraine.

In order to protect interests of Ukrainian agricultural producers and to identify and foresee possible threats and risks we propose to use the possibilities of mechanisms and methodological features of economic security and risk assessment. Our perspective is that the following procedure could contribute to determining the sequence of steps, necessary for preventing possible crises and risks. In addition, it guarantees to improve evaluation mechanisms of economic risks in terms of administrative decisions.

SUBJECTS AND METHODS

The object of our research is the process of economic security managing and risk assessment of the agrarian enterprises. The aim of the present work is to analyze the safety components of the mechanism of competition policy realization at agricultural enterprises, and to conduct a complex assessment of the economic security components of market activities of producers of main export sector with further determination of market activities priorities, and to demonstrate the methodological approaches to threat assessment and risks security of production and sales.

RESULTS

The importance of competitive advantages of certain economic entities and the country as a whole is emphasized in the context of Ukrainian integration with the international economic space and globalization features of this continuum. Competitive policy as a component of economic policy, with the help of the system of state measures contributes to the formation of a competitive environment and ensures protection and support for participants. The possibility to achieve the strategic objectives requires keeping to priority stages of their realization. One of such items is the formation of protective instruments of destructive influence grading of risks and raising the level of economic security.

As is well known, the market dictates conditions to its subjects as far as conjuncture and prices changes are concerned. Consequently, Ukrainian market of agricultural products which is presented by trade groups, based primarily on cereal crops, fruit, eggs [Main economic indicators of production..., 2015] that provide the highest level of profitability and enable enterprises to increase export capacity. Unfortunately, there is a lack of products of animal origin in the existing trade offer. It can’t but affects the achievement of scientifically based consumption standards and Food Balance, while the public policy goal is "the creation of organizational and economic conditions for the effective development of the agricultural sector on the basis of unity of economic, social and environmental interests of society to provide the population with qualitative, safe, affordable foodstuffs and to supply industry with agriculture raw materials " [The strategy of development..., 2013].

The increasing competition forces to react quickly and to improve the existing management decisions and results to strengthen safety components of the mechanism of risk management and its rating. A successful enterprise, which specializes in manufacturing agricultural production operates in terms of certain risk groups, which are both common to all industries and have differences depending on the specialization and concentration

of production. It should be mentioned that both the competition and economic risks and dangers increase in terms of saturation of the market conditions with certain products, and it leads to increasing of economic risks.

Among the risks associated with the specialization of agrarian enterprises it is advisable to identify the following types of risks:

- Frequent change of crop rotation and ecological technologies and increasing of general level of risk due to the limited number of crops and the effect of weather-condition factor (mainly for grain producers);
- Business risks, including instability of prices and exchange fluctuations [Ulyanchenko, 2014];
- Unjustified market oversaturation with the commodity offer and inefficient use of raw material resources, that is typical for the grain industry;
- Imbalance of Ukrainian engineering market [Ivanishin, 2012] and undeveloped logistics infrastructure [Ivanishin, 2011];
- Legislation imperfection, particularly in the tax and customs spheres;
- Incompleteness of land reform.

Origin of risks, factors of their influence, the possible effects and, as a result, methods and ways of their overcoming and decreasing of their level appear according to each type of risk. We believe that it's of vital importance to distinguish safety components, focusing on methods receiving and applying the information to protect from unfair competition, to increase requirements for accounting analytical component of information security at all levels. Price risks being the security element of mechanism of competitive policy realization at the agrarian enterprises, have a huge impact on their economic security. After all, both in terms of the machinery, fertilizers, protection tools and fuel rise in price, and lack of appropriate infrastructure and logistics capabilities heads of small and medium enterprises are forced to cut costs. As a result, it affects the quality of products, releasing the workers, and sometimes causes closing a business.

It is important to ensure the economic security of the country, and in particular - its food security in the context of raising of production rate and aggressiveness of the global economic system. The ability of the competitive advantages of the enterprise to withstand the factors of external environment indicates that the competitiveness of enterprises is a complex of industrial and financial, intellectual and human abilities of the enterprise, which provides it with a firm and competitive positions [Artemenko and Melnik, 2010].

We have found in the process of examining the condition status and trends of Ukrainian agricultural market that without state regulation and institutional approaches to the implementation of the competitive policy the agrarian enterprises cannot prosper. There is a number of factors which are characterized by the imperfection of the complex mechanism of development strategy realization. And one of the most significant, in our opinion, is factor of safety. We should determine the strategic issues of the agrarian sector and highlight the priorities of its development only with the help of preventive steps.

Potential of the agricultural sector of Ukraine makes the realization of ambitious projects to expand sales markets and increase its competitiveness possible. However, there are several items that must be considered in terms of strategic guideline formation of separate sector development and interstate cooperation. Nowadays grain production is considered to be one of the most successful branches of the agrarian sector of Ukrainian economy in terms of gross output growth and exports, providing more than 60 million tons of gross harvest and 30 million tons of grain exports per year [The state statistics service of Ukraine...].

Per capita consumption of grain is much higher than the norm. Grain domination in the structure of the large-scale commercial producer holding companies, which are exporters of grain, allows us to make a conclusion about the necessity of formation and development of economic security, risk assessment and justification of economic decisions. There are certain determinants, which in short or long term, can limit the effectiveness, create a dangerous situation, appear in the specific types of risks and cause risks which can lead to disastrous situations.

The key role in assessing the level of agricultural enterprises competitiveness plays the size of their land Bank and volumes of investment that are not always reflected in high quality of products. So, in grain production, about 40% of foodstuffs are sold for export in the form of raw grain. This fact doesn't assist the improvement of social infrastructure of country territories, increasing the employment and, in our opinion, is threatening in terms of the country. Moreover, it slows down the development of small and medium agrarian business, depletes and contaminates the soil, delays the development of animal husbandry.

Economic security is the state of the national economy, which allows to resist internal and external threats, to ensure high competitiveness in the global economic environment and which characterizes the ability of the national economy to keep the sustained and balanced growth [About approval of Methodical recommendations..., 2013].

The economic security of the agricultural sector is defined as a sustainable state of development of the agrarian sector of the economy where the minimum needs of the society in the relevant agricultural products, raw materials for other sectors of economy, including social needs, food security and the formation of insurance reserves in case of force majeure under the influence of internal and external threats are satisfied. The key criteria are defined in the areas of maintenance of the existing potential and abilities for self-development, taking into account the characteristics of the agricultural sector which is based on the formation of economic security in connection with social stability provision [Sabinski, 2015].

The main characteristics of economic security of grain producers coincide with the given characteristics. However, we need to take into account the individual conditions of functioning, relationships, competitiveness, opportunities to prevent and protect internal and external dangers and risks. Therefore, a comprehensive assessment determines the main advantage of the research status and development opportunities of economic security. It provides for the monitoring of indicators for all functional components. An important element for the grain industry, according to our research, is systematic and complex formation of strategic directions, priority of development, opportunities and threats. The identification of risks and their prevention is the key to success.

A risk assessment methodology of participants of the national grain market must include the impact of global competitiveness and appropriateness of corporate responsibility [Lukin and Solomakha, 2011]. This would prevent the negative trend in cereal production; certain grain producers are extremely sensitive to price volatility both on the grain and on the formation of the resource potential for projecting the next harvest (fuel, fertilizer, equipment, logistics and infrastructure availability).

The definition of strategic guidelines of economic security at the country level involves the use of a multiplicative form of the integral index with the procedure of standardization of indicators and thresholds, justification of the weighting factors [Kharatishviliet. al.].

The indicators in the grain producer activities are calculated according to statistical reports, their definition and as well as their use cause no problems, whereas the identification of indicators in the spheres of identifying and evaluating of risks is more challenging. In addition, complex system of obstacles should be taken into consideration, in particular, the branch imbalance of agriculture, activities in the grain market of large producers, which leads to country unemployment, demographic crisis. The overwhelming majority of monoculture production leads to an unbalanced diet.

In the context of contemporary challenges of economic instability, it is important to formulate the methodology for the assessment of threats and risks in the sphere of production and realization of grain crops, as part of the economic security from the point of view of influence of the industrial development in the financial, social and environmental aspects of economic systems.

The development of grain farming, primarily by large commodity producers, is based on the demand and the monetary effect. This approach should not be based on marketing agriculture, which has specific characteristics in determining the structural characteristics of necessity to grow the product.

We should consider the position of stakeholder assessment and interested parties management, forming a conceptual scheme of the methodology of threats assessing and security risks in production and marketing. We propose to consider this approach in the context of the State target program of development of agrarian sector of economy for the period up to 2020 and to include a State program "Grain of Ukraine 2020".

The globalizations of modern economy and export opportunities of the certain grain producers have a negative impact on the general development of countryside areas. We have arrived to the conclusion that the main reason for this statement is the lack of state influence mechanisms on the rational use of agricultural lands, on their concentration within a single commodity, environmental stress, demographic security, lists of social responsibility of business and so on. Monopolistic activities of large agricultural holdings leads to danger of effective functioning of the state agricultural potential with the help of future, not necessary and not scientifically proved, from our point of view, a strategy of unlimited increase of grain production.

Large-scale enterprises that have export opportunities and get foreign exchange earnings in terms of exchange rate currency fluctuations operate steadily and profitably. To buy seeds, equipment or components abroad is not a problem for them. Whereas medium and small-scale producers receive income in national currency, so are in trouble. This is a kind of a system crisis, the manifestations of which are observed in the protests of the farmers regarding the VAT taxation, which in itself requires a separate research and feasibility studies. To prevent such risks, farmers can be insured through financial partners' programs and have the ability to regulate the cost and profit. The economic safety complex estimation system of grain producers and their international economic relations include the financial mechanisms improvement to facilitate business. Thus, the financial component of economic security prioritizes the grain producer's economic security.

Threat to the economic safety in the grain industry is price volatility in both domestic and foreign markets, mainly due to the influence of the grain market conjuncture. Defining economic security indicators in this direction are the global production and crops sale, the grain Fund structural use of grain world manufacturers.

The economic security formative factor, we believe grain market development infrastructure and its manufacturers. The river transport development, the powerful elevators construction should be a state priority, whereas today these problems are solved by separate holding company. The infrastructure issues and logistics capabilities development of small producers are generally left unattended and are posed a threat to their performance.

It is required improvement and activity managerial field of grain market participants. Recruiting, training and staff development, a high level of adaptability and flexibility in management, professionalism and the ability to self-improvement and self-realization also determine economic security.

Accounting and analytical and information support, innovation in the informational technologies development as economic security components is also required the study and implementation.

The economic security separate priority of the grain sector, consider the rational use possibility of seeds' wastes for the needs of Ukraine's energy independence formation and development. The modern grain production volumes allow to obtain raw materials to support the activities of the enterprises on fuel briquettes manufacture (straw, stalks, cobs of corn for the solid fuel production, seed corn for biofuel). Continuous monitoring requires a grain production balance with the preventing risk aim of environmental and economic risks.

The issue in question requires innovative directions of Ukraine's grain sector development. The active Agro holding structures definitely indicate the modern techniques usage and technologies in production, storage and grain crops sale. The development innovative direction of the agricultural logistics system, which involves the development of infrastructure component in terms of ensuring producers of grain by warehouses for products storage, construction and highways repair and access roads, the railway transport improvement and the its potential maximum usage, the river transport slow development. All of these components will provide progressive return on investment and reduce losses. However, the government in collaboration with the scientists using their own institutions, through the involvement mechanism of public-private partnerships and priorities economic security must monitor, control and regulate the producers' activities and to establish effective strategy and grain farming tactics.

DISCUSSION

One of the regulation key tools of the agrarian market is the prices system and agricultural products pricing, which determines the next priority to the economic security of the grain sector. Through the taxation and regulation, government funding, increase in intervention Fund, it is possible to shape economic security and producers' stability. In particular, article 9 of the Law of Ukraine "On state support of agriculture of Ukraine" stipulates that the intervention Fund for each object of state price regulation may not be less than 20% of their annual domestic consumption for the preceding marketing period [9]. However, the purchases volume of grain crops in 2015 was about 2% of the gross harvest and 35% of domestic consumption, contrary to the law.

The potential resource of Ukraine forms industry development trends and identifies opportunities to strengthen their strategic prospects. From a position of economic security export opportunities create certain threats to the individual businesses effective development, which is manifested in the decrease of their yield and the economic interest reduction in the grain production. The strategy formation of grain farming development should be based on the food security needs of the country, to protect the producers' interests with a view to ensuring their economic security, as well as the need to consider the business development social component, especially large-scale business entities.

At the state level it is necessary to develop strategy of grain industry development, which will include the export grain strategy, a mandatory element should be economic security. This integrated approach will ensure the productive forces development, to improve competitiveness and will open further opportunities for the grain export as a raw resource, but will also enhance the investment attractiveness of the mining sector will increase the power export of grain products with increased added value.

The state support system reform with an emphasis on small farmers, the land reform completion and the state enterprises reform is declared as the main priorities in the "agrarian sector development strategy 3 + 5". Five main directions are the markets' development, organic production and niche crops, rural development, irrigation and food safety [Strategy of development of agrarian sector "3 +5"]. In terms of implementation increases the security importance, because access to foreign markets has not only advantages for national producers, but also facilitates entry into the country cheap, poor quality products, has the market due to low purchasing power of the population and demographic crisis. Thus, there

is a threatening situation the loss of competitive advantages, and that underscores the measures importance of security and counter negative phenomena.

The Ministry of economic development and trade of Ukraine has announced the grain export strategy development with the investment Fund Western NIS Enterprise Fund (WNISEF) support in the framework of the program: «Ukraine leadership Program, promoting exports, attracting investment and development» (ULEAD) [Bulge]. The Fund provides practical assistance in the methodological development and practical recommendations and consultancy services for the development and implementation of export promotion policy, in particular in the public-private partnerships field. It is noted that the existing potential are linked with the relevant challenges that in our opinion, emphasizes the need to focus development of the export grain strategy in the ensuring the economic security direction of producers and the industry as a whole.

The threats definition, hazards, risks rationale will contribute to the grain farming stabilization, because it stands as a tool to support export producers, especially middle management, which will form the development rural areas potential, thereby ensuring the social policy implementation in the village revival sphere by creating jobs, infrastructure development.

A key step in the development of such strategy, we consider a systematic approach to the its elements implementation formation. We must consider the current realities in the globalization of economic relations and especially transnational cooperation opportunities, including financial, grain products potential consumers, and the price changes dynamics, the fluctuations factors and market conditions in the world grain market.

The problematic issues for grain crops producers is also a land and its quality, which do not always correspond to the declared. Enterprises with land banks of over 100 thousand hectares are not always interested in preserving and improving the soils' quality, monoculture in their rotation is a danger from the point of view of cultivation agro technologies of ecological production that can be manifested in social dissatisfaction and which leads to unpredictable consequences. Such scenario, in our opinion, should also be considered in the list of dangers in the elements system formation for the realization of the grain export strategy.

To meet the domestic grain market challenges, as primary in determining the export policy priorities, include: the other countries initiatives interception for the entire chain from production to grain sale, accompanied by the deformation (through the state subsidies mechanisms) and competitive environment in the world grain market; the destabilization

of the internal pillars of Ukraine's food security, which will increasingly depend on the motives of economic expediency manufacturers and traders (companies, the foreign capital share which is constantly growing) to expand the supply to the foreign market and their ability to influence the price dynamics of food products in the domestic market [Dergaluk, 2010].

The export potential formation danger and grain production development should be considered as currency fluctuations, since the national currency depreciation is beneficial to exporters to sell grain and to get the profits that is diametrically opposite the situation in the strengthening of the hryvnia, so the General state of the economy correlates with the interests of large players on the grain market, leads to the economic security imbalance among the individual producers of the region.

The tax legislation imperfection, increasing tax burden has a particularly negative impact on small and medium enterprises, which in rising inflation conditions, appreciation of the resource component in grain production reduces profitability and threatens the downward trend in production.

Declaring increase in grain production and export in Ukraine is almost no effective system of exchange trade with grain. There is a separate practice of forward contracts, but futures contracts are extremely rare, that can be attributed to the economic security interests sphere, since the futures market is the insurance risk tool, in particular the hedging of commodity price risks.

Thus, Ukraine's grain export policy should be formed by taking into account the position of the producers' economic security, subject to consistency in the implementation of its elements which will help to define strategic guidelines for the industry development.

The economic interests sphere of the business entities entrepreneurial activities is located in plane search promising and business development profitable directions. The identifying and developing process such areas include monitoring of the market situation and capacity assessment. The risk assessment simultaneous carrying out and economic security formation will allow you to make informed management decisions that will allow you to react threats and to apply modern management methods to derive economic benefits.

CONCLUSIONS

One of the main industries that form a significant part of Ukraine's export is agrarian, therefore the potential and monitoring study of its threats and risks determine the development strategy directions. One of them consider the economic security priority of the

grain industry, as it affects the economic systems development with the control mechanism help and risk assessment.

Risk management and the tools use of economic security form the combating concept and preventing threats and hazards. The risks of imperfect resource provision, price fluctuations, competition, liquidity depend on political and economic decisions, and adverse climatic conditions, natural disasters, social conflicts, requires measures of economic safety in order to minimize them, is in active risk management.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

PROBLEMS OF MANAGEMENT AND MARKETING IN THE ENTERPRISES ACTIVITY OF AGRICULTURE

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KEYWORDS

marketing, management, agricultural enterprise, governance, economic efficiency, sales

ABSTRACT

Purpose. The functioning of agricultural enterprises in modern conditions require timely management adaptation to external changes, which in turn necessitate the use of marketing management in production administration and sales. Marketing management in its essence is a set of rules for making decisions which companies use in its activities.

Methods. The theoretical base it is a fundamental tenets of economic theory, marketing, management set out in Ukrainian and foreign scientific works on the development of the agricultural sector, used the following methods: abstract and logical - at a theoretical generalization of the essence and content of marketing management, methods of analysis and synthesis - for detalization of the object of research, economic analysis and comparison - in assessing the key efficiency indicators of the companies in agricultural industry, graphic method - for visualisation of structure of industry;

Results. analysis of the category "Marketing Management" by the foreign and Ukrainian scientists. Based on studied literature it was defined the essence of marketing management, its place and role in the overall company management.

Discussion. Verified the economic efficiency of agricultural enterprises in the context of marketing management. Determined the necessity of the modern approach to management of agricultural enterprises, which embodies the new philosophy of governance and its implementation on a new level.

INTRODUCTION

There is a need for a qualitatively new approach to the management of the production and marketing of goods in the modern business environment for enterprises of Ukraine. It becomes necessary to adapt to the economic situation, and therefore "management philosophy" of the company has to change. There is a need in the market orientation control in such a situation.

In this regard, the improvement of business management based on the principles of management and marketing, providing a strategic approach in business activity carried out by marketing management become a particular urgency. This explains the choice of research and its relevance.

Analysis of recent publications and research

Research of many foreign and ukrainian scientists devoted to the problems of formation and functioning of marketing management. Proceedings many foreign and Ukrainian scientists reveal the problems of formation and functioning of marketing management. An important contribution in research of these problems made the next scientists Balabanov L., Voychak A., Kotler F., Malik M., Ostrowski P., Solovyov I. and others. However, despite the research concept of "marketing management", many aspects remain open.

THE AIM OF THE RESEARCH

The aim of the esearch is the disclosure of the role and place of marketing management in the management of economic activities of agricultural enterprises.

RESULTS

For today it is difficult to operate the agricultural enterprises in a free market. After the removing of agriculture from state subsidies, to he heads of many companies faced with a number of problems. Some of them are: inadequate of structure management, inefficient pricing system, the inability of identifying the real needs of consumers, to organize good communicative, marketing, trade policy, work out well-defined strategy for development. Thereby the using of new methods and approaches are accrued to business management in agriculture.

The construction and implementation of market economic mechanism is important in these conditions, which would provide a dynamic balance between supply and demand, the main component of which should be marketing as the modern management’s foundation of market processes, as determined form elimination of contradictions between production and consumption. Condition of the food market and options of its development requires a new approach to its regulation of new, marketing concepts which are adapted to our conditions.

The first defining agricultural market’s feature of Ukraine should be considered as spontaneous, that is restituted of exterior influencing the disposition of its formation and functioning. In fact, it is still stand in the liberal market model. The transition to market all the countries of Central and Eastern Europe (CEE) began from it. However the CEE renounced it in early 90s and began to use state regulation of agricultural markets, creating the concerned institutions, Ukraine still has no effective means of preventing destructive manifestations of market elements.

The second agricultural market’s feature of Ukraine is its limited "transparency".

The authorities of official government statistics do not solve this problem. The methods of distributing market's information are remaining imperfect. Departmental industrial information centers of agricultural market are not properly functioning, although their task was putting before the branch ministry (Ministry of Agriculture) and ministries of the government's economic bloc in 1995.

The third feature agricultural markets feature of Ukraine is an insufficient institutional providing. The institutions or organizations personification food sectors of agricultural markets and institutions with practical implementation of state support for manufacturers are not established till now. Ukraine should learn the experience of the EU in the agricultural market which allocated more than twenty grocery markets as individual independent objects of specific organization, regulation and government support [Kovalenko, 2015].

The formation of supply and demand has special features that significantly affect the internal structure of these market's products and grocery for agricultural products. It (the structure) is crucial for determination of the market's type, identification of relationships between sellers and buyers, the formation mechanism of pricing. Therefore, the determination of market structure allows estimating of its internal surroundings, specificity of behavior the market's relations, to realize its prediction, to implement the effective public regulatory policy in which the market mechanism would ensure the economic stability of normally functioning entities.

The consensus as for internal structure of agricultural markets has not been formed among the economists yet. Therefore it is appropriated to learn and analyze the structure of market by two criteria: quantitative and qualitative. For the first criterion the quantitative ratio of market is given. The views of researchers on the issue as for this assessment are often not the same, especially because some of them are not fully taken formed relationship between the main market participants to note. There is a perception that the agricultural products' market is a market with a clean (perfect) rivalry in particular.

There are some reasons for such a conclusion, especially if we consider this market, only as sellers market (agricultural producers) firstly, and, secondly, without structuring it into separate subsystems from grocery sign. One of the arguments in favor of this viewpoint, is the fact that the production and supply of agricultural products is realized by the large number of independent from each other producers. And this product is quite homogeneous, which greatly enhances the competitive surroundings among sellers. Each individual producer (seller) can not affect the market's price, as its share as a seller of the total offer is extremely small, and the cross-price elasticity of demand for agricultural

commodities, which characterizes the degree of price changes on products.

If the market of agricultural products considered comprehensively, as a sellers and buyers' market, and as a structured fact of its individual segments (subsystems), which has its own. Inherent market infrastructure, the specificity of this dominant segment (grocery market) type of agricultural products, different value of sellers and buyers and features of the levers of state regulation, so such a viewpoint is hardly fair.

The reason which leads to such a conclusion is that the agricultural producers in the majority are not engaged in harvesting and processing of agricultural products (or engaged, in a small extent), but implementing its entities III agriculture's areas, or are intermediaries, which by their number, as buyers, are far below the number of sellers. The gap in such a quantity is often greatly increased on the local markets by the coming of buyer's monopoly.

At the same time the recycling plants have strong market power because of their relatively small quantity, dictating the conditions of purchases to agricultural products. Therefore, there are some reasons to confirm that the classification of commodity markets, grocery markets have olihopsonichnu structure in Ukraine (in some local markets it has a monopsonic structure), where harvesting and processing companies can implement pricing policy in their own interests, to seek more favorable conditions of purchase to themselves and other. They do not have or have some slight spending for replacing some other sellers. It is important to know that the olihopsonichna (monopsonic) structure of agricultural markets is typical for developed countries.

The successive concentration of production in the grocery, processing and light industry is observed, which is accompanied by decreasing of businesses in these industrial sectors and increase of enterprises. And in food trade dominates, usually The oligopoly is usually dominated in a grocery industry where dominant market's position belong to 2-3 large transnational corporations.

Because of agricultural long term problems existence, due to price inelasticity demand of agricultural products, inactivity of resources in agriculture and typical shifts of supply and demand to agricultural products, the tendency of prices' lowering for agricultural products is composed and incomes of agricultural enterprises as for prices and incomes of economy in general [Andreychuk, 2004].

With increasing demand of agricultural raw materials, caused by increasing demand of grocery products, which it is produced, rising prices on the agricultural products and enterprises area III APC. Under such growth the part of consumer's agricultural commodity prices can be even increased, but the processing companies continue to maintain

the marketing margin at given progress, or even higher level, providing a greater income to themself. Having a market power, they work in the demand's increasing conditions to make the price's rising of agricultural raw material slower than rising prices of grocery market.

It is difficult to operate the agricultural enterprises in a free market's conditions for today. After the removing of agriculture from state subsidies, the heads of many companies faced to the number of problems. Some of them are: inadequate management structure, inefficient pricing system, the inability to identify the real needs of consumers, to organize good communication, marketing and trade policies, to work out a clear strategy of enterprises' development. In this connection the needs of new methods and approaches to business management in agriculture are accrued.

In the grocery complex of the country and its regions the certain contradictions between agricultural producers, processors and manufacturers of grocery products, wholesale and retail trade were formed, which led to the disintegration of the grocery market. Rising costs of grocery goods promotion and, consequently, to increase retail prices and some difficulties in assessing the real needs, scope and structure of demand, product range in retail trade area, development of efficient, adapted to the socio-economic conditions of a market's segment, marketing's strategies of grocery producers and commercial enterprises.

According to the conditions of necessity in the construction and implementation of market's economic mechanism, which would provide a dynamic equilibrium between supply and demand, the main component of which is should be marketing as the foundation of modern management in the market's processes, as the determined form of contradictions' removal between production and consumption [Blyznuk, 2003].

Therefore, the condition of the grocery market and options for its development requires a new approach to its regulation of new, adapted to our conditions marketing's concepts.

In Ukrainian marketing literature the term «marketing management» sometimes is translated as marketing management. This is about control of activities based on the principles of marketing, the usage of marketing as a management philosophy where all the divisions of the company plan and evaluate their activities from perspective of marketing. Thus, marketing management is considered as the management of all functions of the company, all the structural units based on marketing [Kucher, 2012].

Formation of modern marketing management that is able to manage businesses through an innovative model of management will ensure the stabilization and development of agricultural enterprises. Transformations of internal and external environment cause the improvement of the management system of agricultural enterprises based on marketing

management, as marketing has won the key positions in the management of companies at the market.

In modern terms, to provide economic sustainability, agribusiness companies have to improve their own economic activities according to the concept of marketing management, which will help fundamentally change the economic environment its operation and further development. The concept of marketing management of APC (Agricultural Prodaction Complex) provides a transition to active search for market partners as the suppliers of production means and consumers of agricultural products.

The modern situation is characterized by narrowing of the financial base of agricultural production development. There is a possibility of the formation sources reduction of funds of agricultural producers through constant currency fluctuations that led to a record increment in prices of fertilizers, fuel, pesticides and energy, adverse pricing environment of major material-technical products and containment of the prices at agricultural products [Sahac'kyj, 2001].

Therefore, the foreign investment in economy of Ukraine play an important role in development of Ukrainian agrarian sector. Total foreign investment in the agricultural sector in 2015 (Table 1) amounted to 617.0 million USD, representing 1.35% of the total amount of investment in Ukraine. However, it should be noted that in 2015, compared to previous years, there was a reduction of the total investment amount and the amount directed to agriculture sector.

Table 1. Direct foreign investments (equity capital) in economy of Ukraine by type of economic activity, (millions USD)

Indicator	**Direct foreign investments at beginning of year**					
Years	2010	2011	2012	2013	2014	2015
Total	40053,0	44806,0	50333,9	55296,8	57056,4	45744,8
Agriculture, forestry and fishing	680,4	730,7	736,3	728,8	776,9	617,0

Source: www.ukrstat.gov.ua

The research of enterprises activities in agrarian sector showed that the number of farms in Ukraine was 45,379at the end of 2015. It shows a decrease of amount of farms in the reporting period.

The analysis of production of farms during the reporting period (Table. 2) showed that 2015, comparing to the previous two years, is characterized by a decrease in agricultural production, crop production and animal husbandry.

Table 2. Output in Agriculture, (in 2010 prices; millions hryvnya)

Indicator	**2012**	**2013**	**2014**	**2015**
Agricultural companiesTotal	49415	49046	46199	45379
Agricultural products– total	113082,3	136590,9	139058,4	131918,6
including				
crop production	82130,2	103127,8	105529,5	99584,7
animal production	30952,1	33463,1	33528,9	32333,9

Source: *www.ukrstat.gov.ua*

The aims of marketing management are defined and shaped on the base of the overall objectives of the company, including a profit, increment of sales and share on the market. From the standpoint of marketing management the list of aims could be supplemented with the conquest of new markets, increase competitiveness of the company and its products, the successful introduction of new products, improve customer service, increase efficiency, etc. [Kotler et. al., 2006].

Ukrainian agricultural sector has the potential of agricultural production that far exceeds Ukrainian demands. The development strategy of the agricultural sector of Ukraine aimed at building efficient state sector that can meet the needs of the Ukrainian market and take a leading position in the global market of agricultural products [Blyznuk, 2003].

Part of the production Ukraine exports to the EU, Asia and other countries.

Analysis of export of agricultural products has shown that in 2015 there was a decrease in all product groups (Table. 3).

Table 3. Commodity Pattern of exports of agricultural and provisions production[1] (thousands USD)

Commodity code and title by Ukrainian Classification of Commodities in Foreign Trade	2012	2013	2014	2015
Total (code 1-24)	17905603,7	17038805,3	16668953,8	14563144,5
I. Live animals and livestock products	960557,4	1084105,1	1014473,9	823434,9
II. Plant products	9222473,3	8875920,1	8736139,2	7971492,5
III.. Animal or plant fats and oils	4210625,2	3507076,4	3822031,8	3299799,1
IV. Finished food products	3511947,8	3571703,7	3096308,9	2468418,0

Source: www.ukrstat.gov.ua

This is partly due to the recurring restrictions on the supply of Ukrainian meat, dairy and other agricultural products, changes in procedures and rules of customs clearance.

The biggest risks concern the export of Ukrainian agricultural products to the CIS countries. There is a need to diversify products markets. In this regard, it is necessary to search for compensations of losses of traditional markets, which is connected with the expansion of the presence of Ukrainian agricultural products in other segments of the world market, including Europe, Asia and Africa.

Effective marketing management operating system provides a stable and long-term development of the company profit.

Main indicators of agricultural enterprises in Ukraine during the chosen period are positive (Table. 4). However, there is a downward trend in net income, but also the share of profitable enterprises is increasing every year.

Table 4. Basic indexes of enterprises activity in Agriculture[1]

Years	**2012**	**2013**	**2014[1]**	**2015[2]**
Net profit (loss), mln.UAH	26728,4	14925,7	21413,4	102279,0
Enterprises which got net profit				
percentage to total number	78,6	80,3	84,7	88,9
financial result, mln.UAH	33570,1	26186,6	51668,0	127360,1
Enterprises which got dead loss				
percentage to total number	21,4	19,7	15,3	11,1
financial result, mln.UAH	6841,7	11260,9	30254,6	25081,1
Profitability level of all types of activity, %	16,3	8,3	9,3	30,5
Profitability level of operating activities, %	22,8	11,7	21,4	43,1

[1]Data are revised.

[2] Data are preliminary.

Source: www.ukrstat.gov.ua

An important indicator of economic activity is the profitability of major products of crops and livestock. This index in agricultural enterprises mainly characterized by positive tendency except definite kinds of animal products, including cattle (-16.9%), sheep and goat meat (-26.6%), poultry meat (-5, 4%) [www.ukrstat.gov.ua].

Marketing Management focuses on the use of a wide range of events with strategic and tactical characteristics, aimed at efficient business activity and maximum profit. Marketing

business management focuses on fulfilling the major purpose of enterprise - profit, growth sales, increasing the share at the market etc.

To obtain positive results including profits, improve business performance, increase competitiveness, the company must follow such principles as:

- Balance of internal and external development opportunities and their adaptation to the needs of the enterprise;
- Autonomy in exchange of resources between the elements of production and distribution systems of the enterprise and between the enterprise and the surrounding environment;
- Operative correction of the management mechanism in accordance with the changes that have already occurred or is predicted [Ostrovsky, 2006].

The main factors that negatively affect the implementation process of marketing management of agricultural enterprises are:

1) lack of a developed market infrastructure;
2) a small number of skilled workers who organize marketing management in enterprises;
3) insufficient resources required for the functioning of the marketing department of the companies.

The world practice of agricultural enterprises management indicates that the current market management creates the leader with special characteristics that is able to mobilize the initiative and responsibility. A market economy requires new highly qualificated leaders and specialists with new approach, who use modern methods of implementation of industrial and commercial activities, capable creatively solve market problems, promptly adapt to changes in the marketing environment and agriculture and provide profit of the enterprises. However, in agricultural production there is a shortage of managers who are capable in process timely response to market conditions, identify social needs and achieve optimization of profits.

Marketing management constantly needs improvement, because the market is very volatile and it is difficult to identify the correct algorithm of the most successful work of the enterprise; but it is possible to concentrate all efforts on external changes in management without sacrificing enterprise. It is what marketing management is looking for.

The agricultural sector - one of the most perspective sectors of the Ukrainian economy. The biggest part of Ukrainian exports are agricultural products, so it is important to reform of Ukrainian agriculture. Reform of the agricultural sector should be financed with taking into account the principles of the new food policy and international assistance. Should be done improvement of the quality of products, must be done the deregulation and attraction

of international lenders and investors. Also, should be paid attention to further European integration, improving the business climate, improvement of efficiency of enterprises.

It would be reasonable to take into account the experience of agricultural reform in Poland, which is an example of how an integrated policy for rural development and attracting investment can lead to successful modernization of agriculture. European Fund of Development Polish villages is one of the most successful and interesting models of development agricultural territories in the EU, which can be applied in Ukraine. To make farming high-tech and interesting for Ukrainian youth Polish experience in this field is very important.

CONCLUSIONS AND PROSPECTS

The study of agricultural enterprises shows that marketing management occurs when management organization built on the principles of marketing. Also consider marketing management and marketing management indivisible, which also complement each other. This necessitates a new approach to economic management, an approach that embodies the new philosophy of governance and its implementation to a new level. This approach provides the use of foreign experience in management and marketing, its adaptation to the specific conditions of each region and Ukraine in general.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

SOLAR RADIATION AS A SOURCE OF ENERGY USED IN DRYING PROCESSES

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KEYWORDS

drying house, drying, radiation, solar energy

ABSTRACT

The work presents methods of using solar energy in drying processes. The impact of atmospheric conditions on the process of drying is described. The first part of the work contains the description of construction solutions in solar collectors used in agricultural and food processing industry. The second part of the work focuses on solar drying houses used for sewage sludge processing in treatment plants. The work presents the problem of efficiency of solutions of this kind as well as the financial aspect of solar drying houses functioning. The work focuses on solar drying houses, and presents technological solutions used in this kind of plants.

INTRODUCTION

In the past, people dried green material (e.g. grass for animals) to preserve nutritional values of crops and increase the storage period of food products. They dried the material in the open air, which was a lengthy process, and largely depended on atmospheric conditions, in particular, air humidity. Material loses occurred at different stages of drying process:

- during drying: green material loses approximately 50% of its nutritional value during drying;
- in transport: it is impossible to transport 100%dried material to the destination due to its fragility and brittleness; some material will be left in the drying place or on a trailer;
- decay processes: material that has not been dried out properly, stored in the storage room without proper ventilation may begin to decay.

Nowadays, drying is used to preserve green material for farm animals as well as other biological materials and sewage sludge. It is difficult to imagine drying without appropriate technologies in the 21st century. With these technologies, the material is dried using methods that do not allow for losses resulting from decay processes. Moreover, the material retains

more nutritional or calorific values due to shorter drying time. In Poland, solar drying houses at sewage processing plants are becoming more popular. In comparison with traditional high-temperature drying houses, drying sewage sludge in solar drying houses requires much less energy.

Due to various applications of solar radiation, as an alternative source of energy in industry, the main goal of the work was to present the potential of using this source of energy in drying processes.

The scope of the work encompasses:

- characteristic of renewable resources of energy,
- methods of drying biological products,
- types of standard drying rooms,
- modern construction solutions using renewable resources of energy (RRE).

SOLAR ENERGY

1.39 kW/m^2 of solar radiation reaches the border of the earth's atmosphere, with only 45% of the total radiation reaching the surface of the earth. Out of the remaining 55%, 22% is absorbed by the atmosphere, 8% is scattered in the atmosphere, 17% is lost by reflection from clouds, another 4% is absorbed by clouds and 6% is reflected from the surface of the earth. As a result, approx. 1 kW/$m^2$of the total solar radiation reaches the surface of the earth [http://www.zielonaenergia.eco.pl/]. The total solar radiation is the total of the reflected, direct and scattered radiation. It depends on the latitude, climate of the geographic region and air pollution. Direct solar radiation is the radiation that reaches the surface of the Earth directly from the Sun through the earth's atmosphere. Reflected radiation is the solar radiation which, on its way to the earth's surface, meets an obstacle such as a cloud or a building and is reflected. Scattered radiation is the radiation deviated in various directions.

Solar radiation is electromagnetic wave, which may be divided into: ultraviolet radiation (wave length of 150-400nm), visible radiation (wave length of 400-750nm), and infrared radiation (wave length of 750-4000nm).

- Solar radiation reaches the surface of the Earth and induces the following reactions:
- photothermic – solar radiation is transformed into heat energy,
- photovoltaic – solar radiation is transformed into electrical energy,
- photosynthetic – solar energy is transformed into chemical compounds energy, which reaction enables plants to produce oxygen from carbon dioxide [Nowicki J.,1980].

Devices, which operate based on the above mentioned reactions are collectors and photovoltaic cells. Collectors use the photothermic reaction to heat up the utility water or the heating system water. We may distinguish the following types of collectors: pipe solar collectors (vacuum), flat-plate solar collectors (gaseous, fluid, two-phase) and concave solar collectors.

The most important part of the collector is the absorber, which is covered by the glass plate. Solar radiation reaching the surface of the Earth penetrates the glass and falls onto the absorber. The absorber absorbs the solar radiation, and heats up heat exchangers, in which the working medium, such as water, glycol or gas, circulates. The temperature of the working medium may increase by a few to more than ten degrees in a single circulation, and the increase of the temperature depends on radiation intensity. In order to reduce heat losses, insulation materials such as mineral wool or expanded polystyrene are used.

A vacuum collector consists of two glass pipes, one transparent one with a greater diameter, which is the shell and another one, with a smaller diameter, covered with a layer of selective coating, which absorbs the radiation. Between the two pipes is the vacuum, which allows for the decrease of heat loses. With vacuum collectors, the temperature of the working medium may reach 150^{o}C. This allows for using such collectors for steam production, and is a considerable advantage. [Klugmann-Radziemska E., 2008].

A concave solar collector consists of mirrors that concentrates solar rays at the absorber, which at the same time, acts as a heat exchanger, filled with the working medium. The advantage of this solution is high temperature of the heated working medium. However, for the highest efficiency, the collector must follow the movement of the sun, which increases the cost of the installation.

Photovoltaic cells use photothermic reaction for the production of electrical energy. There are different generations of photovoltaic cells. Cells of the 1st generation are most often used in photovoltaic panels. A monocrystalline cell is a homogeneous silicon crystal, which results in high efficiency (22%) of PV panels using this type of cell. However, the cost of production of a PV panel is very high compared to other solutions. Polycrystalline cell has slightly lower efficiency compared to monocrystalline cell. A single cell is made of crystallized silicon. Photovoltaic panels of the 1st generation are widely available on the market. They are reliable but not resistant to shading [http://www.obud.pl/].

Cells of the 2nd generation have very thin active layer. Production of panels of the 2nd generation is much lower than the 1st generation but it has impact on the efficiency (10%) and price. Devices using 2nd generation cells are more resistant to shading and perform well

in tropical conditions, characterized by high insolation. The disadvantage of such solutions is a small number of products available on the market, and very limited choice of panels made from 2nd generation cells.

Cells of the 3rd generation do not have a P-N junction, used in the two previous generations of cells, which distinguishes this generation from the preceding generations. Due to lack of P-N junction, the cell is not capable of working with classical semi-conductors. This cell is produced using the method of overprinting of very thin structure. It is extremely flexible and fully recyclable. The disadvantages are short lifetime and low efficiency.

Uncovered collectors do not have a transparent coating, and they are usually mounted on roofs or walls of buildings. They have lower efficiency and temperature rise than covered collectors. This solution is mainly used by farmers for heating up air in drying rooms. In drying rooms, which use such solutions, green fodder, cereal grain, herbs, wood chips, wicker, grass, energy plants may be dried [Wiśniewski G., Gołębiowski S., Gryciuk M., 1999]. Such a collector may be used as the roofing, preferably painted dark mat colour (e.g. roofing paper, roofing sheet or roofing tile sheets) with a built-in air duct. The heated air is sucked up to the drying chamber by ventilators. The duct is between the roofing and e.g. the plywood or fibreboard, in the appropriate distance from each other [Pabis J., Szeptycki A., 2012].

Axial fun of 1-5kW power, static pressure of 100-300 Pa and air flow rate of 10 000-40 000 m^3/h are most often installed in this type of drying rooms. Depending on the type of material being dried, e.g. cereal grain, it is better to install centrifugal fans, which have the range of operation from low to high static pressure, high power consumption and low air flow rate [Pabis J., Szeptycki A., 2012]. The disadvantage of uncovered collectors is dependence on atmospheric conditions.

Covered collectors, as opposed to uncovered collectors, are equipped with a shell made of glass or a transparent plastic. The most important element is the absorber, which may be made of metals such as steel, aluminium or copper, and the casing is made of insulation material. Ambient air heats at two levels simultaneously, between the transparent coating and the absorber as well as between the absorber and the casing. Due to the transparent shell, the amount of heat flowing to the environment, as opposed to uncovered collectors, is limited. In order to increase the efficiency of the collector, it is necessary to add more transparent coatings.

Such drying rooms are intended for drying small plant-derived materials (herbs, grains, berries and mushrooms). It uses direct heating. Such a construction works like an absorber.

The air flowing through vents between the bottom and the holes located at the upper lid is heated up by sun rays penetrating through the transparent material. Green material is put on a net for the purpose of more effective drying from each side. In order to increase the efficiency of the drying room, an additional fan may be installed below the drying chamber to force air flow. In Polish conditions, the roof may be flat or may have 30^{o}pitch [Marks N., 2007].

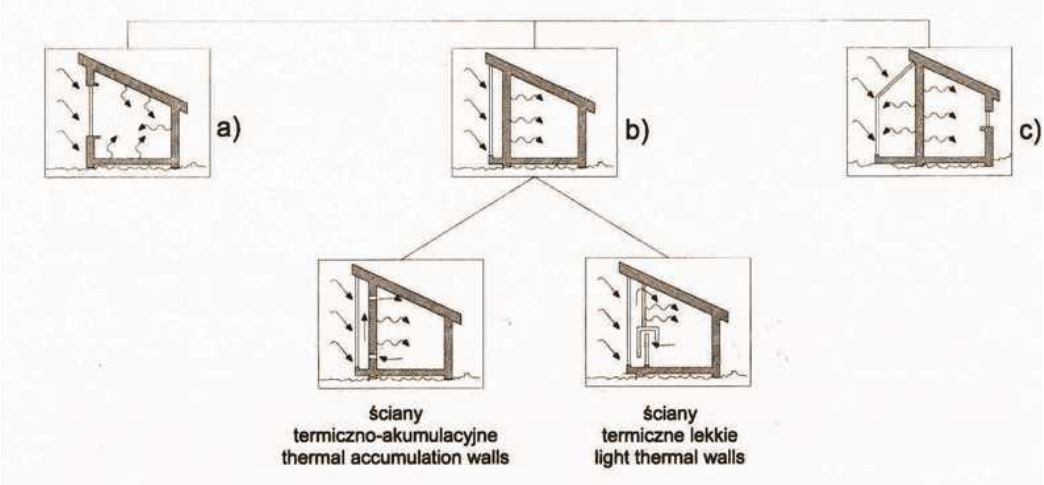

Fig. 1: Solutions related to systems of passive collectors a) direct b) indirect c) complex [Source: Wołoszyn M.A., 1991]

Passive collectors are used in the type of drying rooms presented below, which use the greenhouse effect. Sun rays penetrate through the transparent elements of the drying room, such as windows or glass doors, and are absorbed by the walls inside the drying chamber. The walls releasing heat, heat up the air inside the drier.

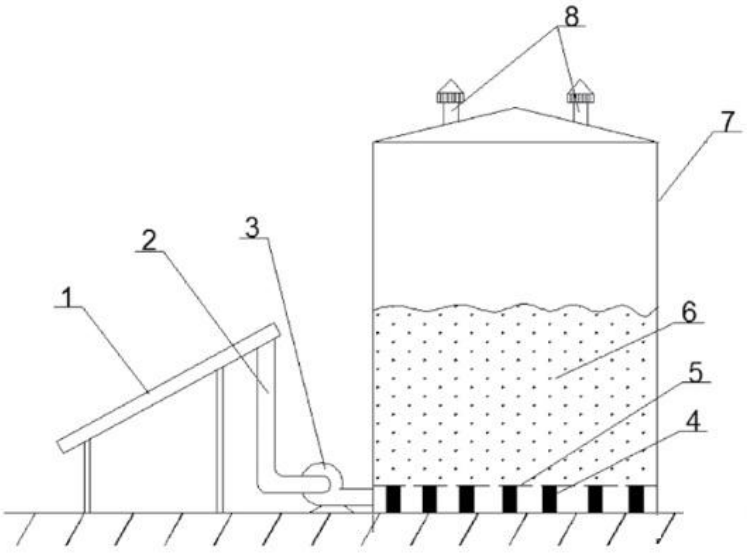

Fig. 2: A diagram of a drying room with an collector. 1 -Collector, 2 - Aggregate channel, 3 – Vent, 4 - perforated sheet floor, 5 - floor support, 6 – Material, 7 – Drying chamber, 8 – Vent [Source: Kopeć A., 2013]

Air collector used in the construction of the drying room, consists of a layer of transparent material (e.g. glass, plexiglass, etc.) and black, insulating material (e.g. black roof paper, foil). In Polish conditions, the absorber should be mounted at the angle of 30° along N-S axis. Material for drying is placed on shelves with perforated (sieve-like) bottom inside the non-transparent drying chamber. Solar radiation falls on the flat-plate air collector. Heated air goes up and through the sieves placed in the chamber and dries the material. Finally, it leaves the drying chamber through the vents in the roof. Such type of the drying house is suitable for drying herbs, mushrooms, berries and fruits [Pabis J., Szeptycki A., 2012].

The principle of operation of a hybrid drying room resembles convective drying rooms. Collectors are not an element of the drying room's construction. The devices are connected by inlet channels which supply heated air directly to drying chambers. Heated air is supplied to drying chambers using ventilators. Ventilators may be supplied by current produced by the photovoltaic panels or from the electrical installation. This drying technology is popular in Poland, and it is used for drying sewage sludge.

The decision regarding the choice of sewage sludge drying method ought to be made based on the location and the size of drying rooms, physical-chemical composition of the material being dried, the final form of the product after drying, final product destination and financial means available.

In Poland, solar drying houses are located in the vicinity of sewage treatment plants. The process of drying sewage sludge is based on renewable resources of energy, thus reducing the outlay for energy.

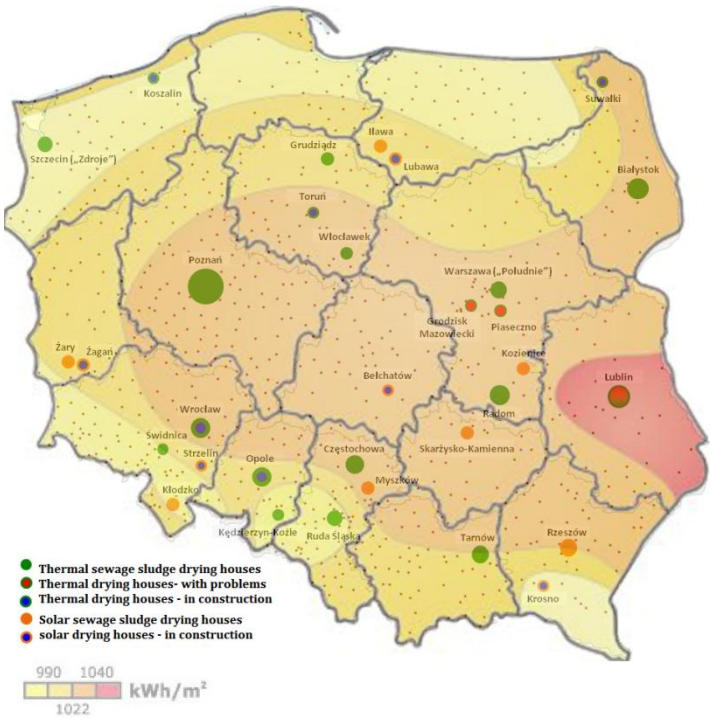

Fig.3: A map of Poland showing the intensity of solar radiation and the location of sewage sludge drying houses [Source:Wójtowicz A., 2012]

The map shows the location of thermal drying houses and solar drying houses and the intensity of solar radiation in Poland. Hybrid drying houses are located, i. a., in Bełchatów, Myszków, Krośno, Kłodzkowo, and solar houses are located in Żary and Lubawa. Poland receives only approx. 1000 - 1100 kWh/m^{2}solar energy [Podogrodzki J., Leszczyński M., 1982]. Solar drying houses operate from spring to autumn. During the winter, they become storage rooms for sludge, as in the winter the insolation is too low, air humidity is too high, and the process of water evaporation takes too long. Summer is the best period for drying, as the insolation is the highest, and the day lasts the longest, thus making sludge drying more intense and cost-effective.

Mechanical drying houses do not depend on atmospheric conditions. However, they consume much more electrical energy for drying out 1m^3 of sludge than solar drying houses. The efficiency of solar drying houses depends on relative air humidity, solar radiation, active area of the drying room, temperature inside the drying room [Mehrdadi N., Joshi S.G., Nasrabadi T., Hoveidi H., 2007].

In drying rooms, greenhouse effect may occur. Direct radiation, reaching the surface of the earth, falls on the roof; some of it is reflected by glass panes and the remaining part is absorbed by the sludge. The sludge heats up from solar radiation and emits heat, which

circulates inside the heating house, resulting in the increase of the temperature inside the house, and accelerating the process of water evaporation from the sludge.

Drying process in solar drying houses predominantly depends on solar radiation, which, in turn, depends on the time of the year.

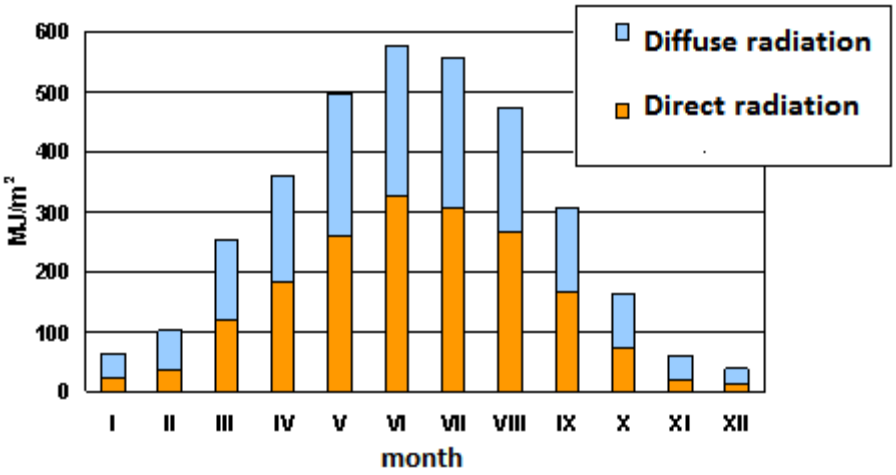

Fig. 4: Monthly amount of radiation received by a flat surface in Warsaw [Source: http://ekologika.pl/]

The graph shows monthly amount of radiation divided into indirect and scattered radiation, which reaches the flat surface in the latitude, in which Warsaw lies. The radiation in various regions of Poland ranges between 3230 MJ/m^2/year and 4310 MJ/m^2/year.

Solar drying houses in the temperate climate zone, in which Poland lies, do not work all the year round. During winter, due to sub-zero temperatures and low insolation, the accumulated sludge may freeze [http://suszarnie.itc.pw.edu.pl/].

Air humidity is a significant parameter that describes the process of drying. Relative humidity of the drying medium depends on air temperature. The potential of humidity reception by the working medium (drying air) is the difference between the dew point and the real relative humidity. In Poland, large diurnal variations in relative humidity may be observed, which do not depend on the season of the year.

High relative humidity during the winter results in lowering the reception of humidity from the sludge by the drying air. In order to increase the reception potential from the sludge, at high air humidity conditions, it is required to heat up the drying medium.

The construction of drying houses is similar to that of a greenhouse, but the house has concrete floor, and is tiled using one-chamber polycarbonate plates (the exception is Strzelin, where the house is built using glass panes). The material used for the construction of the house has good solar radiation transmittance (86%),and low heat transfer coefficient

(3.3W/m^{2}K). The drying process in this type of drying houses involves sun rays penetrating through the roof covered by transparent plates, drawn by the natural colour of sludge [Trojanowska K., 2006].

Sewage sludge technology using solar radiation allows for the continuous work of the drying house. Moist sewage sludge are placed at the entrance to the chamber using loading machines, belt or screw conveyors. At a later stage, a sludge turner gradually transports the sludge to other areas of the drying room. A single passage of this device moves the sludge in the drying room approx. 50 cm, at the same time storing dried out sludge at the far end of the room. Due to the continuous mode of work, the drying house has a higher efficiency in comparison to a drying room with the interrupted drying cycle. [Wójtowicz A., 2012].

Ventilators, which enable air flow inside the room, are the indispensable element of solar drying houses. Such a system of mechanical ventilation was installed in drying houses located in Krosno and Wieruszów. The air is held inside until it reaches the point of steam saturation humidity, and it is replaced with the fresh air. In Kozienice and Żary gravitational-mechanical ventilation (hybrid ventilation) is installed. This system of ventilation works in the following way: the system of ventilation works in a natural (gravitational) way for a longer period of time, i.e. the roof windows open and close automatically, and, additionally, a mechanical system starts working when air exchange is insufficient. The mode in which the system works i.e. natural or mechanical depends on air humidity degree detected by moisture detectors connected to the control system. Circulating air speed forced by ventilators amounts to approx. 1m/s [Malicki M., 1977].

Heat energy from additional sources using cheap fuels is used in hybrid drying houses. The heat is distributed using floor heating system, heating up ventilation air or low-temperature radiators (infrared). There are area-related limitations related to difficult lie of the land in order to make solar-based drying cost effective. Also, additional heat sources are used to dry out more sludge in shorter time [Sobczyk R., Sypuła M., 2012].Drying houses in Kozienice, Żary, Żagań and Lubawaonly use heat from sun rays while drying houses in Rzeszów, Skarżysko-Kamienna and Iława use heat from biogas combustion. Additionally, in Iława, heat pumps are installed as in Kłodzko, Myszków or Strzelin, and in Wieruszów the central heating system boiler is coal-fired [Sobczyk R., Sypuła M., 2011].

Additional sources of heat and reduction of loses by low heat transfer coefficient allow for drying sludge in winter. Drying houses use heat installations such as heat pumps, biogas- or coal-fired boilers. Heated air has lower relative humidity than non-heated air, which

increases the potential of water reception from deposited sewage sludge [Sobczyk R., Sypuła M., 2010].

The disadvantage of heated air is the loss of heat by the construction of the drying room itself, which is light and open. Heat loses are inevitable when floor heating system is used, the cause being the sludge, which acts as insulator. The most efficient method of drying is using additional sources of energy such as biogas-fired infrared radiators. Radiation emitted by radiators and solar radiation have similar characteristics. Biogas combusted in infrared radiators is transformed into electromagnetic waves, which heat the sludge instead of the heating air. According to the principles of thermodynamics, drying effectiveness is higher when warm surface layer of the sewage sludge is blown through by the cold air [Trojanowska K., 2011].

Solar drying houses that use solar radiation as an alternative source of energy have both advantages and disadvantages. The advantages include low energy consumption, using renewable resources of energy, simple construction, no pollution emission, low cost of drying, shorter drying time in comparison with open-air drying, no additional source of heat such as heat oil or natural gas required, automation. The disadvantages include dependence on atmospheric conditions, lack of a system to regulate the parameters of drying process, very long drying time in comparison to thermal drying houses.

SUMMARY

Poland lies in the temperate climate zone, which is characterized by lower intensity of solar radiation in comparison to the countries in tropical or equatorial climate. In this latitude, various technological solutions allowing for solar energy use are applied, with the most popular being solar collectors, which transform solar energy into heat energy, used for heating utility water, and photovoltaic cells, which allow for the transformation of sun energy into electrical energy. The solutions are not only used in industrial establishments or enterprises but also in individual households or detached houses.

In agriculture, solar collectors are used for drying different kinds of agricultural products such as crop grain, power plants, hay, fruits or mushrooms. Construction of some driers is not very complicated and may be done without hiring professionals. Collectors may be mounted on roofs or south walls of buildings.

Solar energy is used in production plants such as sewage treatment plant for the purpose of drying sewage sludge. Sludge drying may be divided into solar – solely based on solar radiation energy, and hybrid – requiring additional sources of energy, such as heat pumps,

biogas or coal. The most effective solution is a hybrid drying house with low-temperature gas-fired radiators.

The cost of such a solar drying house for sewage sludge drying depend on its exclusive reliance on solar energy or use of additional sources of energy.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

THE MICROBIOLOGICAL AIR QUALITY AT THE FRUIT AND VEGETABLE PROCESSING FACILITY

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KEYWORDS

bacteria, fungi, bioaerosol, fruit and vegetable processing facility

ABSTRACT

The aim of the study was to characterize the microbiological quality of air at the fruit and vegetable processing facility. The study was carried out during autumn season in 2016. Air samples were collected in triplicate at three sampling points inside the building and at the one located outside the building. The air samples were collected using a 6-stage Andersen impactor. During sampling, the air temperature, relative humidity and particulate matter were measured. The results showed that the bacterial aerosol concentrations in the measuring points ranged from 467 cfu·m^{-3} to 833 cfu·m^{-3} and the fungal aerosol concentrations in the measuring points ranged from 219 cfu·m^{-3} to 339 cfu·m^{-3}. The lowest concentrations of bacterial and fungal aerosol were observed in the outdoor air. The highest concentrations of bacterial aerosol were observed at production hall and the highest concentrations of fungal aerosol were observed at the room with vegetable and fruit peeler. There were significant differences in the bacterial aerosol concentrations between the measuring points. The correlation between the concentration of bacterial and fungal aerosol and temperature, relative humidity and particulate matter in the air was significant. Bioaerosol concentrations obtained in this study were lower than reference values for bacteria and fungi in the workspaces contaminated by organic dust, recommended by the Polish Panel of Experts of Biological Factors.

INTRODUCTION

One of the factors that can affect people's health is atmospheric air [Dokładna et al. 2015]. In the air, there are ingredients that can create various types of pollution and a serious health problem. Microorganisms in the air, such a bacteria and fungi, can cause infections, immunotoxic diseases and allergies [Karwowska 2003]. Exposure to harmful components of bioaerosols in the workplace can cause many adverse health effects (simple irritation, various toxic and allergic reactions, infections and infectious diseases) [Douwes et al. 2003, Zielińska-Jankiewicz and Kozajda 2003]. The threat from biological factors may be related to the performance of a particular profession and the presence and properties of factor [Dutkiewicz 2004]. The health effects of inhalation of aerosol particles are related to their physical, chemical and biological properties [Więcek 2011].

The susceptibility of ready-to-eat foods to microbial contaminants from the air is variable. The most sensitive for this type of pollution are products characterized by high moisture and nutritional value, which gives an excellent environment for the growth of microflora. Proper organization of work, efficient ventilation and automation of production processes can effectively reduce the number of microorganisms as a result of reduced product contact with the environment [Kręgiel 2006]. Modern technologies of various industries require more and more microbiological air purity and inspire the need for the development of research in this field. As a result of experiments and observations, it was found that the air in the environment of the production line is often a source of secondary contamination of the product. A large number of microorganisms in the air is often the result of poor sanitary conditions of machinery, equipment, ventilation, air conditioning and often insufficient hygiene of production personel [Palka 2007].

The most important sources of air pollution at the production facility are personnel and activities within the production, ventilation, air conditioning and sewage systems. People are the source of the microbial pollution of the air, secreted with saliva in speaking, sneezing or coughing. Some of the droplets of human-made saliva fall on the walls and floors in the wet state, the rest evaporates, and some microbes are suspended in the air. In addition, the head (mainly hair) and hands, as well as clothing (work and protective clothing) and footwear can be the source of microflora emitted by people into the production environment [Palka 2007].

The aim of this study was to characterize the microbiological quality of air at the fruit and vegetable processing facility.

MATERIALS AND METHODS

The study was carried out during the autumn season in 2016. Air samples were collected at the fruit and vegetable processing facility, in triplicate at three sampling points in the building (room with vegetable and fruit peeler, drying room of the finished product and production hall) (pic. 1) and at the one point located outside the building. The facility produces vegetable pastes and fruit jams. In facility, a mechanical ventilation system is used. Measurements were performed during normal facility operations. The air samples were collected using a 6-stage Andersen impactor (model 10-710, Graseby-Andersen, Inc., Atlanta, GA, USA) (pic. 2). The sampler was placed at a height of 1.5 m above the floor or ground (outdoor measurement) level to simulate aspiration from the human breathing zone. A 5- minute sampling period and the flow rate of 28.3 $dm^3 \cdot min^{-1}$ were applied for the

collection the air samples. Bacteria were collected on tryptic soy agar (TSA LAB-AGAR™, Biocorp) and fungi were collected on malt agar (Malt Extract Agar, Biocorp). During sampling, the air temperature and relative humidity were measured using hygrometer Kestrel 4000 (pic. 3) and particulate matter (4.0 μm and 10.0 μm)was measured using dust analyzer Dust Trak II (model 8530, TSI Inc., Shoreview, MN, USA).

The TSA plates were incubated for 1 day at 37°C, then 3 days at 22°C and another 3 days at 4°C. The MEA plates were incubated for 4 days at 30°C, then 4 days at 22°C. After incubation, the colonies of microorganisms were counted. The concentration of bioaerosol was calculated as the number of colony forming units per cubic meter of air ($cfu \cdot m^{-3}$). A primary analysis of the composition of microorganisms isolated from the air was also performed: Gram staining for bacteria isolates and preparations in lactofenol for mould fungi strains.

The results were statistically analyzed using Statistica 12 (StatSoft, Inc., Tulsa, OK, USA). The analysis of variance (ANOVA) was calculated and the significance of differences between means was verified by Tukey's test (α=0.05). Results of the effect of microclimatic parameters (temperature and relative humidity)and particulate matter on the quantitative presence of microorganisms in the air were evaluated using the "r" coefficient of the Pearson's correlation.

Picture 1. Production hall at fruit and vegetable processing facility (*source*: *own elaboration*)

Picture 2. 6-stage Andersen's sampler (*source*: *own elaboration*)

Picture 3. Hygrometer Kestrel 4000 (*source*: *own elaboration*)

RESULTS AND DISCUSSION

The concentration of bacterial and fungal aerosol are presented in table 1. The results showed that the bacterial aerosol concentrations in the measuring points ranged from 467 cfu·m^{-3} to 833 cfu·m^{-3}. The highest concentration of bacteria was observed at production hall (833 cfu·m^{-3}) and the lowest concentration of bacteria was observed at outdoor air (467 cfu·m^{-3}). The analysis showed significant differences in the concentrations of bacterial aerosol between the measuring points. The concentration of bacterial aerosol at production hall was significantly higher than concentration of bacteria in the air of room with vegetable and fruit peeler and outdoor air (Tukey's test; $p<0.05$). There were no significant differences in bacteria concentrations in the air between room with vegetable and fruit peeler, drying room of the finished product and outdoor air (Tukey's test; $p>0.05$) and between production hall and drying room of the finished product (Tukey's test; $p>0.05$). Concentrations of bacterial aerosol obtained in this study were lower than reference values for bacteria in the workspaces contaminated by organic dust, recommended by the Polish Panel of Experts of Biological Factors [Górny 2010].

The fungal aerosol concentrations in the sampling points ranged from 219 cfu·m^{-3} to 339 cfu·m^{-3}. The highest concentration of fungi was observed at the room with vegetable and fruit peeler (339 cfu·m^{-3}) and the lowest concentration of fungi was observed at outdoor air (219 cfu·m^{-3}). The analysis showed non-significant differences in the concentrations of fungal aerosol between the sampling points (Tukey's test; $p>0.05$). Concentrations of fungal aerosol obtained in this study were lower than reference values for fungi in the workspaces contaminated by organic dust, recommended by the Panel of Experts of Biological Factors[Górny 2010].

Predominant groups of bacteria were Gram-positive cocci and Gram-positive rods, which is typical for air. Isolated fungi were represented mainly by *Penicillium* sp., *Cladosporium* sp. and *Aspergillus* sp. – mould reported as typical part of bioaerosols.

In Poland, there are no standards for the microbiological contamination of air at production facilities. However, the bioaerosol concentrations obtained in this study are higher than the proposed values (acceptable by the European Union) for bacteria and fungi in the food industry production areas. In case of bacteria, in two of the examined rooms of fruit and vegetable processing facility (drying room of the finished product and production hall), the obtained concentrations of bacterial aerosol were higher than proposed values for bacteria (600 cfu·m^{-3}). In case of fungi, in all of the examined rooms of fruit and vegetable processing facility (room with vegetable and fruit peeler, drying room of the finished product

and production hall), the obtained concentrations of fungal aerosol were higher than proposed values for fungi (0 cfu·m^{-3}) [Palka 2007].

Food processing facilities should have annual and monthly maintenance and inspection schedules for air conditioning and ventilation, which will allow them to maintain their proper technical and hygienic conditions. It has a huge influence on the quality of air supplied to the premises. Using even the most stringent hygiene rules will not prevent the possibility of microbiological contamination of products, for example by air from ventilation and air-conditioning installations, if it is not properly and constantly cleaned [Palka 2007].

Tab. 1. Concentration of bacterial and fungal aerosol inside and outside the fruit and vegetable processing facility (± Standard Deviation)

	Sampling point	Bacteria [cfu·m^{-3}]	Fungi [cfu·m^{-3}]
Indoor air	Room with vegetable and fruit peeler	472 b* (±14,6)	339 a (±8,6)
	Drying room of the finished product	628 ab (±32,7)	262 ab (±11,2)
	Production hall	833 a (±34,9)	276 ab (±8,9)
Outdoor air		467 b (±31,6)	219 ab (±14,1)

*Means marked with the same letters are not significantly different by Tukey's test (α = 0.05)

Source: own elaboration

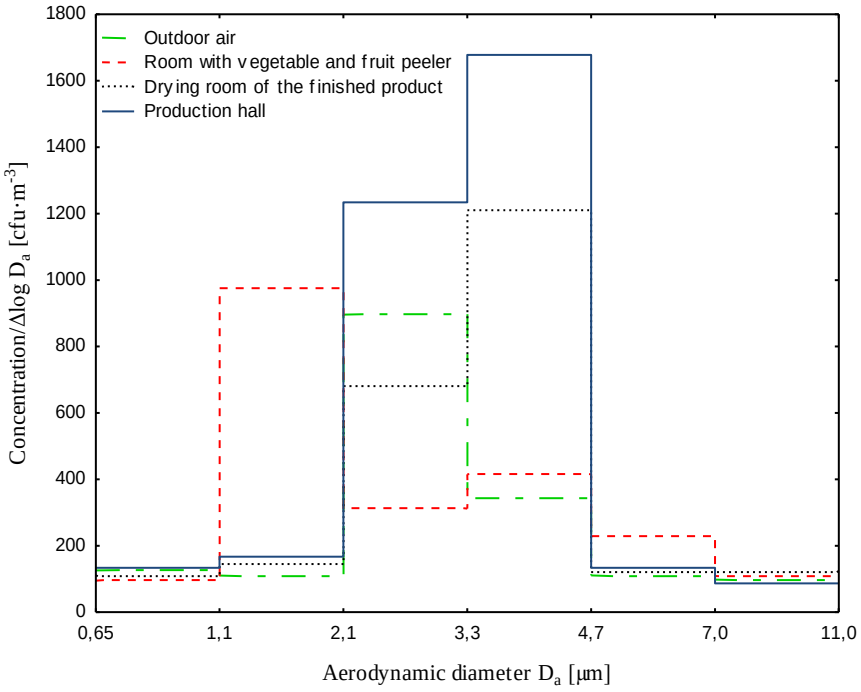
By using a 6-stage Andersen's air sampler, it was possibile to get informations about the size distribution of air microflora in the investigated sampling points (Figure 1 a-b). Based on the analysis of bioaerosol particle size distribution it was observed that the bacteria concentration at production hall and drying room of the finished product had a maximum value in a range of diameters 3.3-4.7 μm. At the room with vegetable and fruit peeler the concentration of bacteria had a maximum value in a range of diameters 1.1-2.1 μm. It shows that these microorganisms can be deposited in the human respiratory tract in trachea, primary bronchi, secondary bronchi and pulmonary bronchioles [Wlazło et al. 2008].

Concentration of fungi at production hall and drying room of the finished product had a maximum value in a range of diameters 3.3-4.7 μm. At the room with vegetable and fruit peeler the concentration of fungi had a maximum value in a range of diameters 2.1-3.3 μm. It shows that these microorganisms can be deposited in the human respiratory

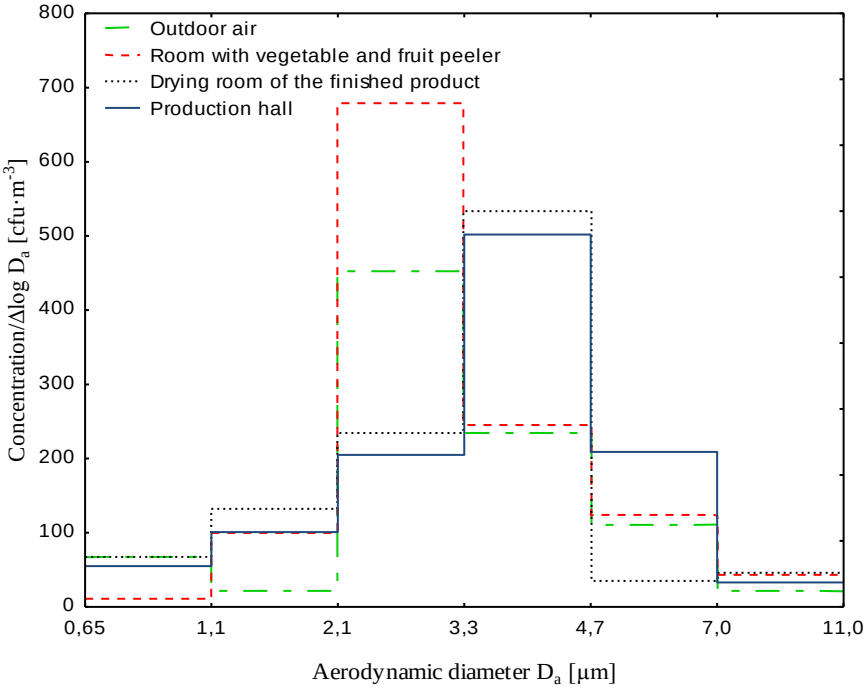
tract in trachea, primary bronchi and secondary bronchi [Wlazło et al. 2008]. Exemplary fraction distribution of bacteria and fungi on cultivation media, isolated from the air by 6-stage Andersen's air sampler, is presented on picture 4 a, b. The aerosol of particles smaller than 5.0 μm remains the longest in the air, while the larger is sedimented. From the point of view of human health, the respiratory fraction (particles below 7 μm), is most important, because these microorganisms can enter the upper and lower respiratory tracts [Breza-Boruta 2015].

Fig. 1. The size distribution of bacterial and fungal aerosol inside and outside the fruit and vegetable processing facility: (a) bacteria, (b) fungi

a)

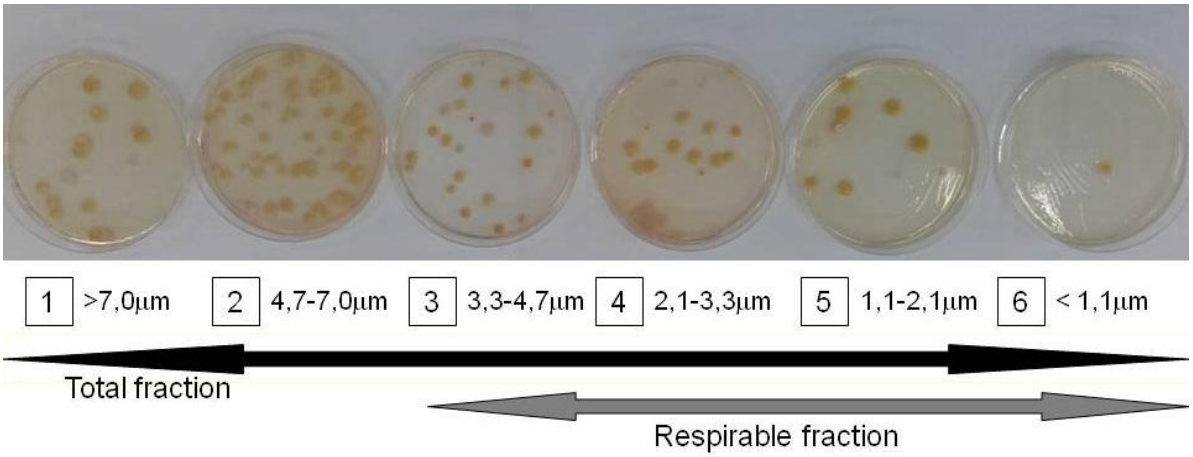


b)

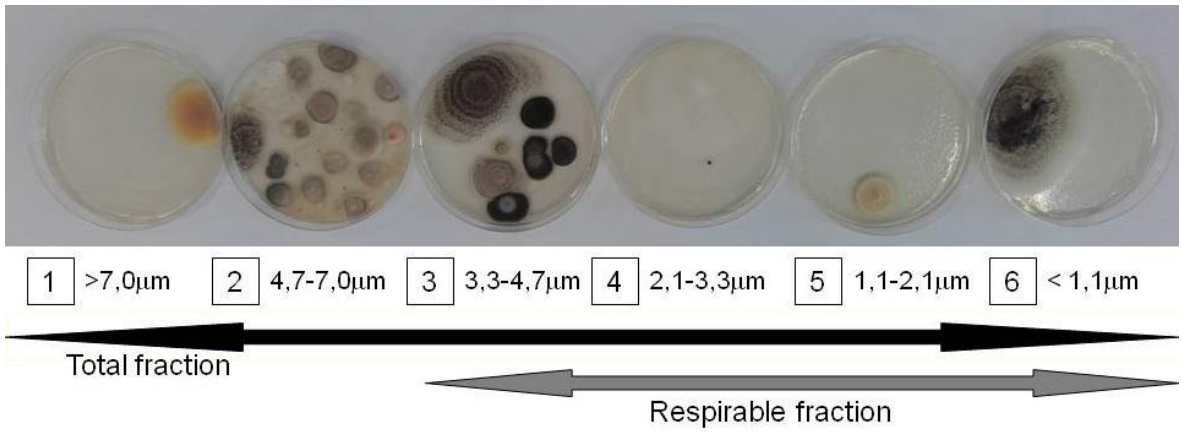


Source: own elaboration

a)



b)



Picture 4. Exemplary fraction distribution of microorganisms isolated from the air: (a) bacteria, (b) fungi (*source*: *own elaboration*)

Microclimate conditions may affect the number of microorganisms in the air [Katial et al. 1997]. Microclimate parameters and pariculate matter are presented in the table 2. Analysis of the impact of the temperature, relative humidity and dustiness on the observed bacterial and fungal aerosol showed that these factors had significant effect on the concentration of the total number of bacteria and fungi in the air. There was a significant correlation between temperature and concentration of bacterial aerosol ($R=0.42$, $p<0.05$) and fungal aerosol($R=-0.63$, $p<0.05$). The analysis showed a significant correlation between the concentration of fungal aerosol and relative humidity ($R=0.33$, $p<0.05$). Analysis showed a significant correlation between the concentration of bioaerosol and particulate matter – fractions 10.0 and 4.0 μm (for bacteria accordingly: $R=0.77$ and $R=0.44$, $p<0.05$; for fungi accordingly: $R=0.66$ and $R=0.61$, $p<0.05$). It proves that the increase in the concentration

of dust affects the increase in the concentration of bacteria and fungi in the air at fruit and vegetable processing facility. Particulate matter is thought to be serious health threat on account of its easy access to respiratory tracts. Harmful health effects can be caused by particulate matter chemical composition, but also by other components, which are absorbed on its surface. Among them, there are microorganisms that potentially influence human health [Frąk et al. 2014].

Tab. 2. The values of microclimatic parameters and particular matter in the indoor and outdoor air at the fruit and vegetable processing facility

Measuring point		Temperature [°C]	Relative humidity [%]	Particular matter – fraction 10.0 µm[mg·m^{-3}]	Particular matter – fraction 4.0 µm [mg·m^{-3}]
Indoor air	Room with vegetable and fruit peeler	16.7	64.8	0.073	0.072
	Drying room of the finished product	21.2	34.5	0.085	0.079
	Production hall	18.4	42.7	0.099	0.091
Outdoor air		11.0	32.1	0.061	0.061

Source: own elaboration

CONCLUSIONS

1. The lowest concentrations of bacterial and fungal aerosol at fruit and vegetable processing facility were observed in the outdoor air (accordingly: 467 cfu·m^{-3}and 219 cfu·m^{-3}). The highest concentrations of bacterial aerosol were observed at production hall (833 cfu·m^{-3}) and the highest concentrations of fungal aerosol were observed at the room with vegetable and fruit peeler (339 cfu·m^{-3}).
2. In case of bacteria, in two of the examined rooms of fruit and vegetable processing facility (drying room of the finished product and production hall), the obtained concentrations of bacterial aerosol were higher than proposed values for bacteria in the air of the food industry production areas. In all of the examined rooms of fruit and vegetable processing facility (room with vegetable and fruit peeler, drying room of the finished product and production hall), the obtained concentrations of fungal aerosol were higher than proposed

values for fungi in the air of the food industry production areas. It proves that the current ventilation is not efficient. There is a need to apply a more efficient mechanical ventilation system.

3. Predominant groups of bacteria and fungi were typical for the air at this environment.
4. Considering employee safety, the concentrations of bacterial and fungal aerosol obtained in this study were lower than reference values for bacteria and fungi in the workspaces contaminated by organic dust, recommended by the Panel of Experts of Biological Factors.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

THE FAUNA OF HELMINTHES *TRICHURIS GENUS* (*NEMATODA, Trichuridae)*, PARASITIZINGIN SHEEP ON THE TERRITORY OF POLTAVA DISTRICT, UKRAINE

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KEYWORDS

Fauna, trichurises,sheep, extensiveness, invasion intensity, Poltava district, morphometry, peculiarities of morphology

ABSTRACT

For the first time the fauna of nematodes – causal agents of trichurosis ovis on the territory of Poltava district has been described. The extensiveness and intensityof animals' infestation with trichurises have been determined; the peculiarities of the given invason progress, both mono- and mixed invasions together with other kinds of helminths and protozoa, parasitizing in the gastro-intestinal tract of sheep, have been described.

Theaverageextensiveness of sheep trichurises invasionconstituted 31,81 %, its intensity was 106,7±10,08 of eggs in 1 g of faeces.

It has been pointed out that trichurosis progressed in the form of mixed invasions (58,56 %) in three-component associations together with strongilates and nematodiruses (16,92 %),, and also eimeries and stongilates (16,92 %).

The fauna of sheep trichurisesisre presented by one kind– *Trichuris ovis*, for which the characteristic morphometric parameters have been singled out: the dimensions of males and females; the structure and dimensions of spicules, spicular vagina; the structure and dimensions of vulva in females, the structure of the cephalad, uterus in females, and gonadal eggs.

INTRODUCTION

At present, sheep-breeding, as well as live stock farming on the whole, acquiresa consider able social and political importance. It is a universal branch which supplies mutton, by-products, milk, wool, and takes the third place in the world as to statistical indicators. The animals' genetic resources vary bo thin their number and quality. For example, Great Britain lead sinthea mount of breed varieties (51), then come France (38), Italy (30), Bulgaria (25), Greece (14), Portugal (12), Spain (11), Poland (11).) Every year Europe sells 450 000 tons of meat, produces 55% of cheeses made from sheep milk (the elite varieties from France, Italy, Spain). 92% of districts – the producers of sheep wool, are located in Europe[2, 12, 14, 15].

Under the conditions of the Ukrainian market the branch is continuing toex ist because, be sides food products, raw materials for medical and pharmacological industries, sheep produce wool – the alternative natural raw material to synthetic fibers, and in future during the aggravation of energy crisis it can take the leading place in satisfying the demands of the mankind [5, 22, 23, 25].

Invasion diseases including helmin thoses are one of the reasons which to so meex ten trestrain the development of sheep breeding branch. The impac to fanthropogenic factor on epizootic situation concerning nemato doses of sheep threatens to increase the number of the population of parasitic diseases' causal agents in the environment and raise the risk of infesting animals and also humans with them. Thus, the problem of helmin thoses in sheep has general biological importance [1, 3, 4, 6, 7, 9, 16].

The analysis of special literature shows that under sheep pasture management practically all animal sintheherd are in fested with various kinds of helmiths, mainly the causal agents of gastro-intestinal nematodoses, including trichurosis, in which the nematodoses of genus *Trichuris* Schrank, 1788, are the causal agents. It is known that evenundera low intensity of invasion definite physiological shifts causing pathological and immunological processes take place; such changes result in considerable economic losses because of not getting enough products from the animals [8, 13, 17, 18, 24].

Taking into account the experience of Ukrainian and for eign researchers who have studied the functioning of classical parasitic systems in sheep organism it should be noted that many questions of the regional peculiarities of parasitoses' epizootic manifestation, trichurosis in particular, are considered to be studied not enough.

In connection with all the above mentioned, it is necessary and timely to study the modern state of sheep trichurosis causal agents' fauna in various climatic and geographical regions of Ukraine, in particular, in Poltava district.

MATERIALSANDMETHODS

There search has been held during 2016-2017 on the basis of the laboratory of the Department of parasitology and veterinary-sanitary expert examination of the Faculty of veterinary medicine at Poltava State Agrarian Academy under the conditions of sheep breeding farms with different forms of property on the territory of Poltava district.

During parasitological examination of the stock the main indicators of sheep in festation with the causal agents of trichurosis, other helminthes, and protozoa have been the extensiveness and intensity of the invasion. Helminthoovos copy of faeces samples has been

held using Mac Master'smethod (1995) in the following way:4 gof faeces were im bedded with 30 mlof flotation solution into a small glass; then the mixture was carefully stirred and the flotation solution was added to the volume of 60 ml; a pipette was dipped into the center of the small glass and the ready fecal suspension was sampled; Mac Master's chamber was filled and settling down was held for not less than 20 seconds; the sample was investigated under the microscope; the number of eggs was counted and the received number was multiplied by 50. The final numeric meaning corresponded to the number of eggs in one gram of the faeces. 349 coproovoscopic examinations were held altogether.

With the aim of determining trichurosis causal agents' fauna, 28 intestines of various age groups of sheep have been examined; the sheep have been taken from slaughterho uses of Poltava district. During the work the methods of the complete helminthological enterotomy of the different intestines' compartments have been used according to the methods of K.I. Skriabin (1928). Small and large intestines were dissected separately. The dissection was made with scissors on the side opposite to the frill attachment; the bowels were filled in with water and washed with it. The conten to feach in testines compartment was examined following the methods of sequential washing. Deep scraping from mucous lining was made, and it was also examined according to the methods of sequential washing. The found helmiths were put into test tubes and fixed in 70° spirit. Helminth identification as to their species wash eldusing the identifier of V.M. Ivashkin and others (1989). The found trichurises were cleared using lactophenol in order to study the peculiarities of their morphological structure. Attention was paid to the body form, the structure of cephaladandcaudal tip, the structure of spicule and spicular vagina in males, the vulva structure and the presence of various formations, the uterus egg structure in females.

The biometry wash eldusing the object micrometer, ocular micrometer, and microscope magnifying × 100, × 400; micro-photo-filming was made with the help of the digital camera to the microscope MICRO med 3Mpix (China). The statistical processing of experimental in vestigations' results was held by determining arithmetical mean (M) and its error (m). Altogether 326 helminths have been investigated –out of them 104 males and 222 females.

Investigation was conducted in accordance with the «General ethical principles of experiments on animals» (Ukraine, 2001) and in compliance with the international requirements of the European Convention «On protection of vertebrate animals used for experimental and other scientific purposes» (Strasbourg, 1985).

RESULTS

The results of the research have established that the average extensiveness of sheep trichurosis in festation under the conditions of the farms in Poltavaregi on constituted 31,81 % having the invasion intensity of 106,7±10,08 eggs in1 g of faeces. The indicators of trichurosisinvasion intensity fluctuated from 50 to 650 eggs in1 g of faeces.

It has been mentioned that trichuros is as a mono-invasion has been registered more rarely – in 41,44 % of cases. More often associated invasions have been revealed (58,56 %), in which trichurises were a component of the association with helminths (nematodiruses, strongilates, strongyloides, moniezies) and protozoa (eimeries) (table 1).

Table 1. The spreading of trichurosis in the composition of associated invasion in sheep under the conditions of the farms in Poltava district

Kinds of parasite combinations	n	%
Two –component:	21	32,31
Trichurises+eimeries	1	1,54
Trichurises +nematodiruses	2	3,08
Trichurises + strongilates	17	26,15
Trichurises + strongyloides	1	1,54
Three-component:	29	44,62
Trichurises +strongilates+nematodiruses	11	16,92
Trichurises + eimeries +strongilates	11	16,92
Trichurises + strongilates + moniezies	3	4,62
Trichurises +strongilates+strongyloides	1	1,54
Trichurises + eimeries +nematodiruses	3	4,62
Four-component:	15	23,07
Trichurises + eimeries +nematodiruses+strongyloides	5	7,69
Trichurises + eimeries +moniezies +strongyloides	7	10,76
Trichurises +strongilates+strongyloides+ eimeries	3	4,62

More often three-component associated invasions have been registered (44,62 %), where trichurises parasitized in sheep organism together with the causal agents of strongy latoses and nematodyrosis (16,92 %), and also with the causal agents of eimeriosis and strongylatoses (16,92 %). Morerarelytwo-component (32,31 %) and four-component (23,07 %) mixed invasions have been revealed. For example, out of the two-component

associated invasions, trichurosis in sheep progressed more often together with strongilates (26,15 %), more rarely – together with nematodiruses (3,08 %), strongy loides and eimeries (1,54 % each). Out of four-component as sociated invasions trichurosis progressed more often together with eimeries, moniezies, strongyloides (10,76 %) and together with eimeries, nematodiruses, strongyloides (7,69 %), and more rarely- together with stongilates, strongyloides, and eimeries(4,62 %).

With the help of helminthological examinations it has been established that helminth fauna of genus *Trichuris*, parasitizing in sheep on the territory of Poltava district, is represented by one species *Trichuris ovis* Abildgaard, 1795, and the sheep infestation according to the data of post-mortem dissection constituted 100 %.

The morphological examinations have established that the body form of adult trichurises' forms, parasitizing in sheep, has the structure characteristic for the helminthes of this family. The frontal part of the body, where esophagus is located, is considerably thinner and longer than the rear one. The body is of white color (Fig. 1). The given species of trichurises has been isolated from the cecum cavity of sheep.

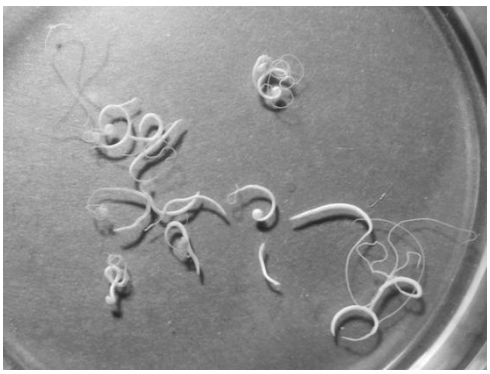


Fig. 1. The external appearance of trichurises' adult forms isolated from sheep intestines.

Microscopically the cephaladhasa cuticulardilation on both sides looking like a "skirt". The mouth is simple, lips are insignificantly expressed (Fig. 2). The esophagus is long and thin. The caudal end is much thicker than the cephalad. The anus is located sub terminally (Fig. 3).

Fig. 2. The structure of the cephalad *T. ovis*, × 400

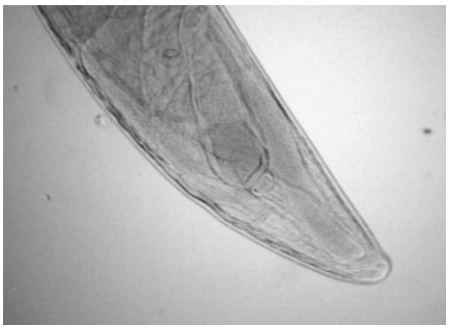


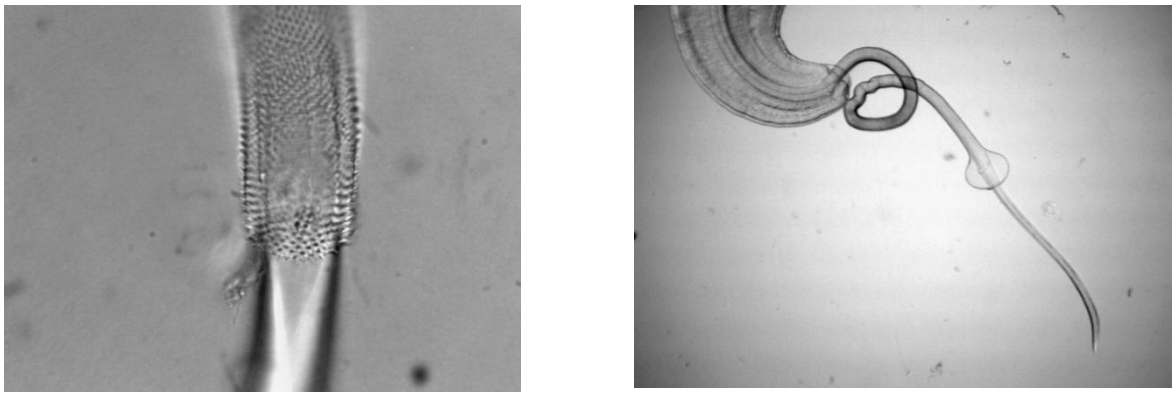
Fig. 3. The structure of the caudal end *T. ovis*, × 100

The body length of males constituted 49,9±1,4 m monthe average, and the length of the thin cephalad was longer (33,7±1,0 mm), than that of the caudal end (16,2±1,1 mm) in correlation 2,2 : 1 between them. The width of the cephaladwasal so less (214,6±6,9 mkm), than that of the caudal end (718,6±10,7 mkm) (table 2).

Table 2. Morphometric indices of the males *Trichuris ovis* (n=10)

Indices	M±m	min–max
Body length, mm	49,9±1,4	42–56
The length of the cephalad, mm	33,7±1,0	28–38
The length of the caudal end, mm	16,2±1,1	10–22
The correlation of the cephalad to the caudal end	2,2 : 1	–
The width of the cephalad, mkm	214,6±6,9	184,1–250,2
The width of the caudal end, mkm	718,6±10,7	658,1–766,1
The length of the spicule, mkm	6826,7±46,5	6589,3–6953,2
The width of the dilation of the spicule proximal end, mkm	135,8±1,4	129,9–141,2

There is one spicule, gubernaculumis absent, the spicular vaginaiscy lindrica land is covered with short spinules of equallengthonits all in vagination space (Fig. 4 b). The spicular vagina has a characteristic dilation at its end (Fig. 4 a).



a, × 400 b, × 40

Fig. 4. The peculiarities of the caudal end structure ♂ *T. ovis*

The spicule length constituted 6826,7±46,5 mkm, on the average, and the width of the dilation of its proximal end was135,8±1,4 mkm.

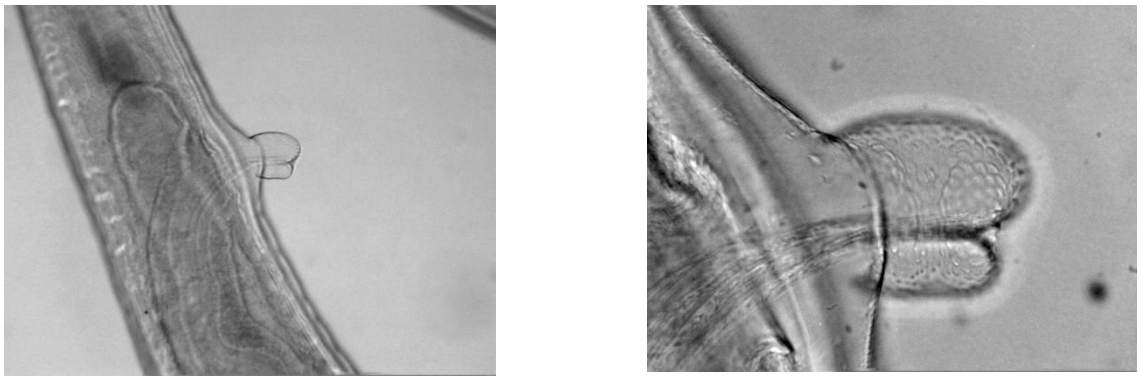
The body length of females constituted 57,0±1,8 mm, on the average which is12,46 % larger, than that of males, and the length of the cephalad, like in males, was larger (42,2±1,6 mm), than that of the caudal end (14,8±0,6 mm) in correlation of 2,9 : 1. The width of the cephalad in females constituted 197,7±8,2 mm, and that of the caudal end was913,0±23,6 mm (table 3).

Table 3. Morphometric indices of females and gonadal eggs of *Trichuris ovis* (n=10)

Indices	M±m	min–max
Body length, mm	57,0±1,8	47–68
The length of the cephalad, mm	42,2±1,6	35–50
The length of the caudal end, mm	14,8±0,6	12–18
The correlation of the cephalad to the caudal end	2,9 : 1	–
The width of the cephalad, mkm	197,7±8,2	165,2–243,9
The width of the caudal end, mkm	913,0±23,6	818,3–1043,3
The width of the vulva tubercle, mkm	159,1±10,1	106,4–197,2
The length of the longer labium of the vulva tubercle, mkm	96,2±5,3	60,5–126,4
The length of the shorter labium of the vulva tubercle, mkm	78,5±2,7	65,9–92,1
The length of gonadal eggs, mkm	73,2±1,0	68,8–80,3
The width of gonadal eggs, mkm	38,1±1,4	30,4–44,7

The vulva is located on the protruding over the body surface tubercle having oval-cylindrical form, and unbent toward the caudal end; the tubercle visually consists of two parts, separated by the genital opening (Fig. 5 a). Morphometrically, the width of the vulvatubercle constituted 78,5±2,7 mkm, where the dimensions of the longer labium were 18,4 % (96,2±5,3 mkm) larger than those of the shorter one (78,5±2,7 mkm).

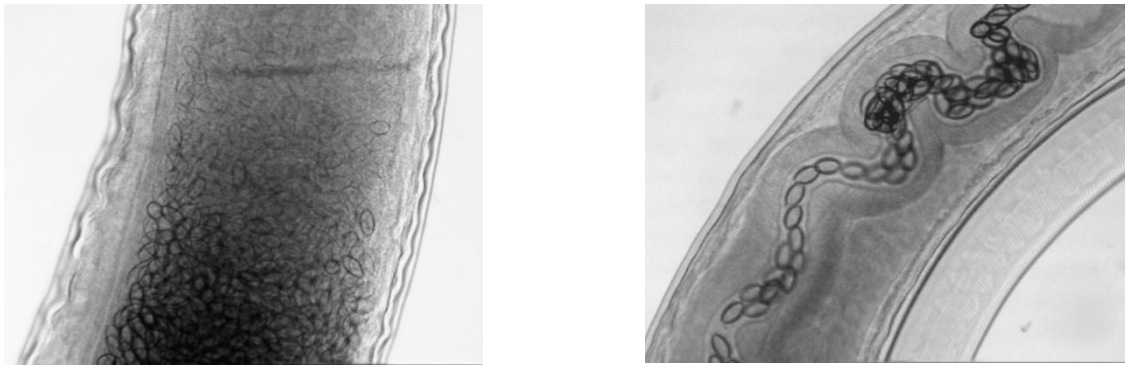
The vulvatubercle, as well as spicular vagina is covered with corymbose spinules (Fig. 5 b).The caudal end is insignificantly curved.



a, × 100 b, × 400

Fig. 5. The peculiarities of vulva area structure ♀ *T. ovis*

The uterus fills all the rear part of its body, and it is filled with a large number of eggs (Fig. 6 a). The uterus becomes thin nernear the vulva and looks like in testines lemniscuses; there are uterus eggs in its cavity placed in one or two lines, sometimes there are empty sections (Fig. 6 b).



a, × 100 b, × 100

Fig. 6. The peculiarities of the uterus section structure ♀ *T. ovis*

The uterus eggs have a characteristic structure: they are oval with short caps on both ends and immature (Fig. 7).

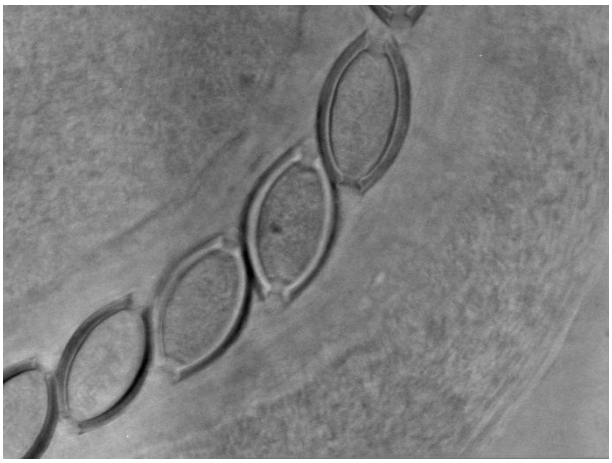


Fig. 7. The peculiarities of uterus eggs' structure *T. ovis*, × 400

The length of go nadal eggs constituted 73,2±1,0 mkm and their width was 38,1±1,4 mkm (table 3).

Thus, the is olated adult forms of *T. ovis* species have diagnostic morphometric indices which are characteristic for the given species.

DISCUSSION

While studying the fauna and spreading of the causal agents of sheep trichurosis, for the first time the data concerning the given causal agent on the territory of Poltava district have been obtained. It can be noted that the species composition of nematodes is represented by one species *Trichuris ovis* having the extensiveness and intensity of invasion 31,81 % and 106,7±10,08 of eggs in 1 g of faeces correspondingly. More often the given helminthos is progresses in animals like an associated invasion together with nematodes, cestodes, and eimeries. According to the scientific investigations [20], heldin all the climatic and geographical zones of Moldova, trichurosis is a spread invasion under which the degree of sheep infestation can reach 100 %. At the same time, the average degree of sheep infestation in the Pre- Caspian region of Russia [21] is 39,3 %, and the trichurises fauna is represented by two nematode species – *Trichuris ovis*и*Trichuris skrjabini*. Never the less, there is little information concerning associated progress of trichuros is together with other causal agents of helminthoses and protozooses of sheep gastro-intestinal tract. That is why the data obtained supplement already existing results of research concerning sheep trichurosis.

Themorphologicalresearchhasestablishedthatmaturetrichuriseshaveacharacteristicbodystructure – the frontal end is thin and long, while the rear one is short and thick, which confirms the data, obtained by the scholars [11]. Nevertheless, the correlation between these parts of males' bodies (2,2 : 1) and females' (2,9 : 1)has been analyzed by us for the first time. Also the data concerning the width of the cephalad and caudal end in females (197,7±8,2 and

913,0±23,6 mkm) and males (214,6±6,9 и 718,6±10,7 mkm), received with the help of morphometricin vestigations, have been isolated into the characteristic diagnostic index for the first time. It has been shown that there are two kinds of cuticulardilations on the helminthes' cephalad, and they are well noticed under the microscope. The data have also been present edconcerning vulvadilation and its metrical parameters.

According to the data of many examinations [19, 10], the structure of *Trichures ovis* eggs is specific, and it is possible to define the species considering this structure. For example, the eggs of *T. ovis* have the following parameters: 0,073–0,078 × 0,035–0,037 mm (on the average 0,0725 × 0,036 mm). Morphologically, the eggs contain wide capson the heads, and soft, thin, transparent first outer membrane, which envelops the whole egg, and as mooth surface of the second outer membrane; the caps are short, without the expressed neckon the poles, they are not connected with the embryo, but slightly protrude from the egg membranes. According to the results of our research, the length of *T. ovis* gonadaleggs constituted73,2±1,0 mkm, and their width was 38,1±1,4 mkm, which only partially corresponds to the data described above.

CONCLUSIONS

In the conclusion it should be noted that trichurosis in sheep is aspread in vasion on the territory ofPoltavadistrict, the average invasion extensiveness was 31,81 % and its intensity was 106,7±10,08 eggsin1 gof faeces. More often trichuros is progressed in sheep as an associated invasion (58,56 %), in which trichurises were a component of the association with helminthes (nematodiruses, strongilates, strongyloides, moniezies), and protozoa (eimeries). Besides, three-component associated invasions (44,62 %) have been registered more often. Post-mortem examinations have revealed that the fauna of genus *Trichuris* helminthes which parasitize in sheep on the territory of Poltava district is represented by one species of *Trichuris ovis*, and the invasion extensiveness was 100 %. The new data concerning morphometricindices of *Trichuris ovis* in malesand females have been received; these data have diagnostic and general biological importance.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

HISTOLOGICAL CHANGES IN KIDNEYS OF RABBITS AFFECTED BY SPONTANEOUS ENCEPHALITOZOONOSIS

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KEYWORDS

encephalitozooncuniculi, rabbits, pathological changes, kidneys, brain, microstructure, dystrophic-necrobiotic changes, leptomeningitis, encephalitis

ABSTRACT

The article deals with characteristic pathological changes in the kidneys and brain of rabbits suffering from spontaneous encephalitozoonosis. The aim of research was to study the histo-pathological changes in rabbitswhich died as a result of encephalitozoonosis. In the kidneys of rabbits with acute course of the disease, structural and functional complex of disorders develop as a result of necrobiotic-dystrophic processes in the system of tubules against the background of hyperemia. In rabbits with asubacute course, dilatation of proximal convoluted tubules prevailed in the kidneys with atrophy of epithelial tubular cells, focal tubular necrosis and desquamation of epithelial of the direct tubules with moderate growth of connective tissue in the interstices. Serous-hemorrhagicleptomeningitis in the brain of rabbits and focal purulent encephalitis develop as a result of the reproduction of the spores of encephalitozoonosis pathogen.

INTRODUCTION

Nowadays microsporidia cause significant problems (economic losses) in such sectors as farming, silkworm filature, rabbit breeding and and in human medicine - as one of the most common parasites among HIV-infected patients [Desportes I. 1985;Modigliani R., 1985; Asmuth D. M. 1994]. According to research scientists, the risk of infection exists for children because their immune system is not yet fully formed.

Intensive study of microsporidia began in 1922 when J. H. Wright and E. M. Craighead identified E. cuniculi pathogen in rabbits affected by “nerve palsy”[Wright J. H., Craighead E. M. 1922]. Subsequently spontaneous invasions caused by E. cuniculi were recorded in rabbits, mice, rats, muskrats, guinea pigs, hamsters, shrews, goats, sheep, pigs, horses, domestic dogs and cats, foxes, and various other exotic predators and primates [Shadduck J. A., Pakes S. P. 1971; Wasson K., Peper R. L. 2000].

Encephalitozooncuniculiisa small obligate intracellular parasite of the Encephalitozoon species which belongs to microsporydia and primarily affects the central nervous system (brain and spinal cord), and also kidneys, liver, spleen, heart, lungs and eyes of rabbits.

Invasion of animals is caused by E. suniculi or other kinds of Encephalitozoon, known as encephalitozoonosis.

Using molecular biological and immunological tests, three genetic types of *Encephalitozooncuniculi* are currently distinguished:

- I (“rabbit type") established in rabbits, mice and people in Switzerland;
- II (“murine type") established in mice, black-brown foxes, not established to date in human body;
- III (“canine type") established in dogs and in people with immune deficiency in the US and the UK [Didier P. J. 1998].

Seroepidemiologic data indicate that rabbits are a host for E. cuniculi type I [Ditrich O. 2011], and the source of infestation in other animals and humans.

Infestation of animals can occur in several ways:

- alimentary - the use of feed or water contaminated with spores of E. suniculi;
- fetal - spores can penetrate through the maternal and fetal placenta and invade the fetal organs;
- contaminative - inhalation of dust from spores during mechanical cleaning of cages or other procedures associated with dust in places where animals are kept [Snowden K. F. 2004, Künzel F. 2008].

Once in the body of a rabbit, the spores penetrate the epithelial cells of the intestine, and then are moved around by the circulation in the internal organs - kidneys, liver, lungs, heart, and brain. The kidneys and the brain are often places for localization of parasites [Cox J. C. 1979, Csokai J. 2009]. E. cuniculi spores can also be found in the anterior chamber of the lens, but solely as a result of transplacental transfer from mother to fetus [Wolfer J. 1993, Baneux P. J. R. 2003].

Intracellular propagation of parasites in the tissues of the nervous system and parenchymal organs cause severe destruction of cells and lead to inflammation in the body in response to the presence of pathogens. [Harcourt-Brown F. M. 2003] First lesions in the kidneys, liver, and lungs show within 30 days after infestation, no changes to be seen in the brain. At the same time, three months after infestation, most visible lesions are observed in the brain. At this time the parasites are absent in the lungs and liver. The heart may also be affected, though usually to a lesser degree [Cox J. C. 1979].

The investigated corpses indicate exhaustion of sick animals. The mucous membranes are anemic. Characteristic lesions are found only in the areas of intensive parasitism of the pathogens. The first lesions usually appear in the kidneys, liver and lungs. Approximately

three months after infestation with the encephalitozoonosis agents, significant histological changes are observed in the kidneys and brain; lesions in the lungs and liver are reduced and parasites cannot be revealed. The heart may also be affected, though usually to a lesser degree. In spontaneously infested rabbits, E. cuniculi spores are observed more often in the kidneys than in the brain.

E. cuniculi spores can be found in the kidney tissues after about four weeks of the invasion. At the initial stage of the disease, a significant number of spores in the epithelial cells of the kidneys or freely floating in direct renal tubules are identified. Thus focal granulomatous interstitial nephritis is observed. In the later stages of the disease interstitial fibrosis without detection of the pathogen in the the foci of inflammation is revealed [Csokai J. 2009].

Affection of the central nervous system and penetration of E. cuniculi spores into the brain occurs after their replication and development in the kidneys. Parasites can be detected in the brain tissues after about eight weeks after the invasion [Cox J. C. 1978]. Spores mainly develop in the brain, brainstem, spinal cord, cerebellum and vestibular nuclei, causing multifocal granulomatous meningoencephalitis [Csokai J. 2009]. The soft and arachnoid membranes of the brain are almost always affected. The granulomas contain lymphocytes, plasma cells, glial cells and are often necrotic in the center [Harcourt-Brown F. M. 2003]. The degree of brain lesions do not necessarily correlate with clinical signs. Thus, in 71 spontaneously infested rabbits vestibular nuclei were affected only in 37.5% of the cases [Csokai J. 2009].

Transplacental transfer of pathogens leads exclusively to eye disease and E. cuniculi spores can be detected in the anterior capsule of the lens. Cataract and facoplasticuve it is are registered at the same time, they are characterized by penetration of various inflammatory cells (granulocytes, macrophages, and giant cells) into the lens. Plasma cells and lymphocytes penetrate into the iris and ciliary body [Giordano C. 2005].

During the chronic course inflammation is mostly not observed In the other organs of rabbits.

Encephalitozoonosisin rabbits in our country is little known disease, therefore the study of pathological and morphological changes during this disease is of vital importance. The aim of our study was to investigate histological changes in the kidneys in rabbits suffering from*encephalitozoonosis [*Kotsyumbas H. I., Levytska V. A. 2014*]*.

MATERIALS AND METHODS

A disease in rabbits characterized by neurological manifestations was detected on the rabbit farms located in Khmelnytskyi region. The animals were placed under clinical observation. ELISA revealed that among the farm rabbits extensiveness of invasion by encephalitozoonosis reached 34%. Autopsy studies were conducted on animal carcasses, kidneys and brain were sampled for histological examination. Pieces of kidney and brain were fixed in 10% neutral formalin, dehydrated and embedded in paraffin. [6] The performed histosections were stained with Hematoxylin and eosin, examined under a microscope. Light microscopy and microphotography of the histological slide were performed with OLYMPUS CX 41 microscope and OLYMPUS C-5050 camera.

RESULTS

Pathological-anatomical and histological investigation

Pathological-anatomical changes in the brain and kidneys were detected in 23 dead rabbits, which manifested clinical signs of encephalitozoonosis, with varying degrees of severity, for a month or more. Presence or absence of certain lesions was established through examination of at least two separate sections of each organ.

Pathomorphological changes in the brains of rabbits

Once the braincase removed, the brain revealed marked redness and thickening of the pia mater. One also observed marked strong injection of blood vessels of the meninges, cerebral cortex and cerebellum (Fig. 1).

Fig. 1 Severe congestion of meninges in rabbits

In some rabbits small pockets of softening of the brain tissue were observed. The substance of the brain was of pastose consistency, with well-defined vascular injection, the boundary between gray and white matter clearly expressed.

Histologically sagittal slices of the brain of rabbits showed morphological changes in pia mater, cortex, less so in cerebellum. Pia mater was affected almost in all the examined corpses of rabbits, where inflammatory changes were clearly observed. Prevalently, veins and capillaries were dilated, full of blood, sometimes calibrated with different degrees of degeneration of the walls. Destruction of capillaries showed, so did swelling of endothelial cells; some capillary walls were destroyed, space around them filled with erythrocytes (Fig. 2).

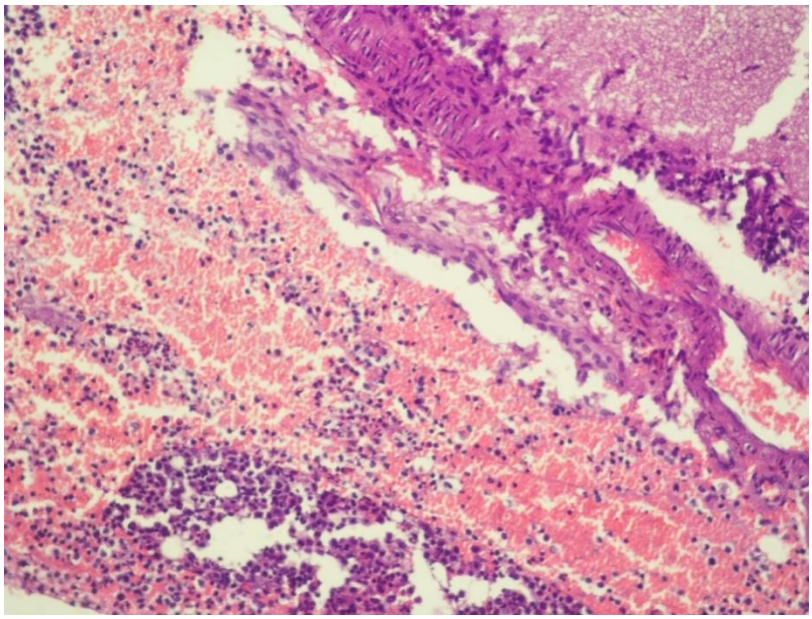

Fig. 2 Veins and capillaries expanded, full of blood, with different degree of degeneration of the walls(Hematoxylin and eosin stains, oc. 10 lens 20)

In some areas of leptomening it is showed slight permeation of membranes with serous exudate was observed. In other areas was noted a significant loosening of connective tissue fibers of pia mater and permeation of serous exudaterich in lymphocytes, erythrocytes, and leukocytes, indicating the development of sero-hemorrhagicleptomening it is(Fig. 3). In most affected areas of mater a marked change in the substance of the brain was detected.

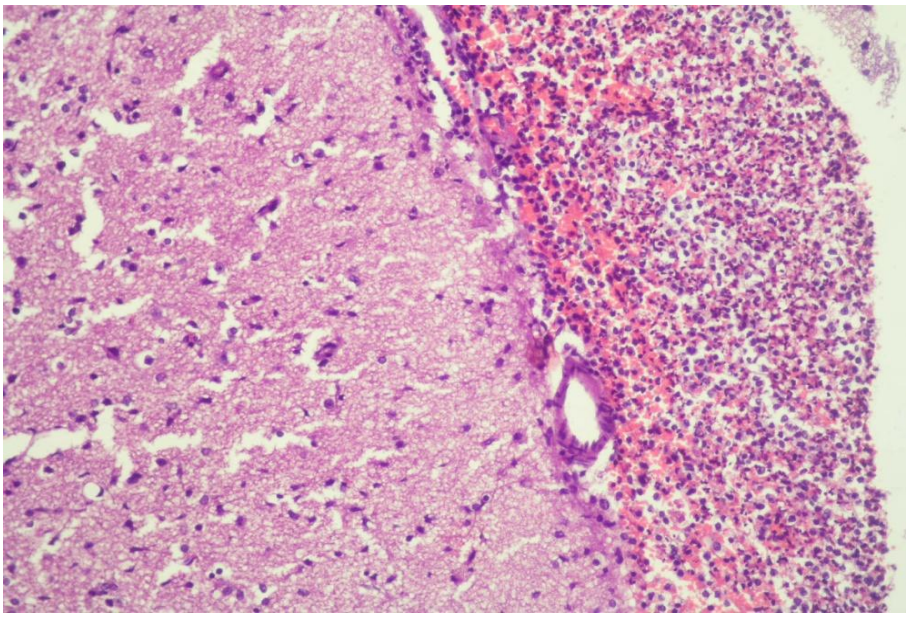

Fig. 3 Meninges intensely impregnated and erythrocytes leukocytes (Hematoxylin and eosin stains, oc. 10 lens 20)

Hemodynamic disturbance in a plethora of capillaries, stasis and *diapedesishemorrhages* were established In the cortex. Hyperemia, pericelar and per vascular edema were observed in almost all the parts of the brain by using optical light. The microstructure of the neurons was not of the uniform type. Well contoured neurons with moderately colored cytoplasm and rounded nucleus located in the center of the cell were detected (Fig. 4).

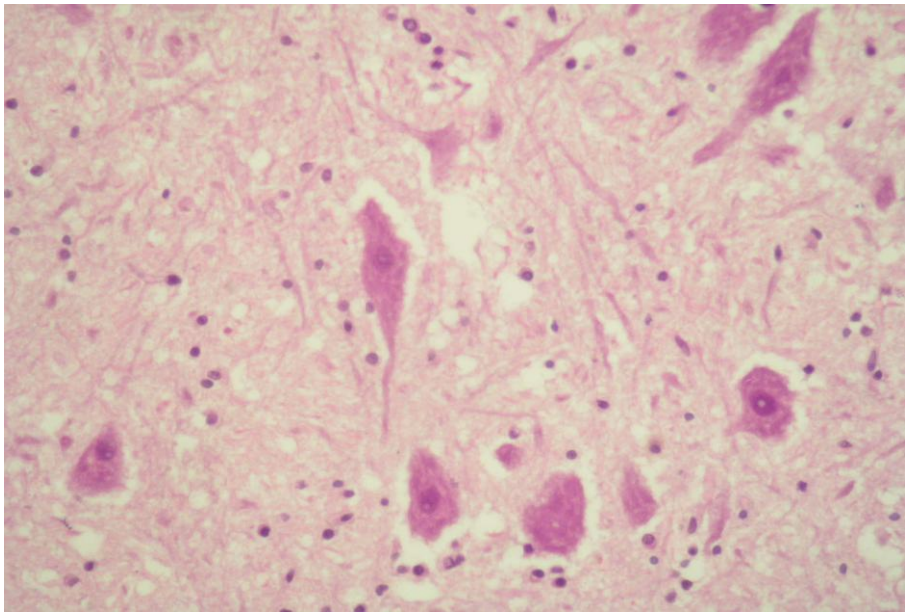

Fig. 4 Neurons of one of the nuclei of the medulla oblongata (Hematoxylin and eosin stains, oc. 10 lens 40)

At the same time, neurons in a state of acute tumefaction with eccentrically placed nucleus were observed both in the cortex and medulla of the brain. The contours of these neurons were barely noticeable, but the neuroplasm was clear, thenuclei shifted to the periphery of the cell often had blurred outlines. A significant decrease in chromatin was observed in the neurocyte nuclei due to its partial destruction. Slightly stained nuclei had a deformed shape. Among such neurons cells were detected where the dyeconcentrated in the peripheral zone and cytoplasm in the form of thin threads, giving a foamy appearance and clear contours to the cell (Fig. 5).

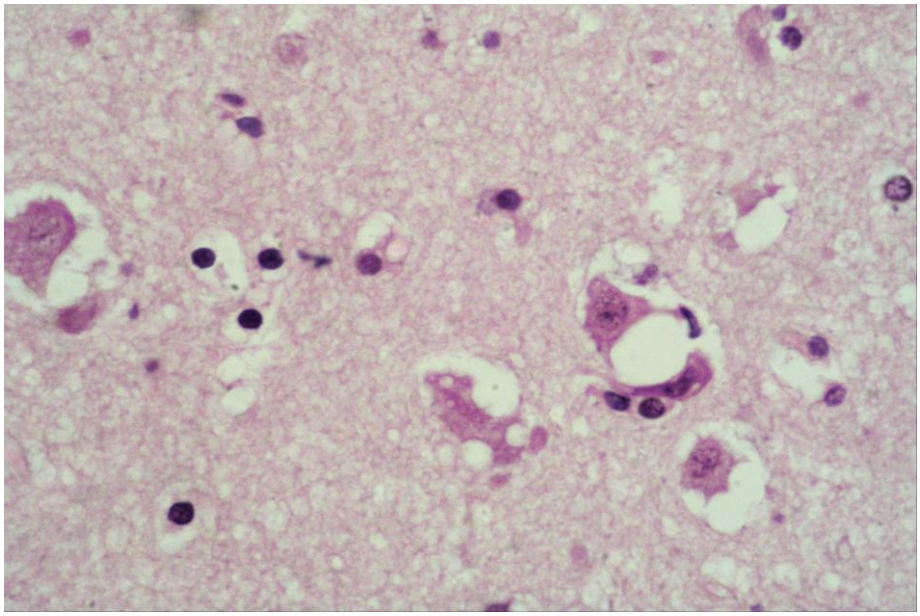

Fig. 5 Pericellularswelling. Total lysis of the nucleusand cytoplasm of the neuron (Hematoxylin and eosin stains, oc. 10 lens 100)

The nuclei of such neurons were reduced. Cells in a state of dissolution were detected. They were mostly of irregular shape, with blurred contours, where not only chromatophil substance was destroyed, but dissolution of the shell and core occurred. Cells in a state of coagulation were detected, which were characterized by a decrease in size.

Accordingly, with the nerve cells damaged, chromatolysis of the tigroidsubstance begins with the perinuclearzone. At this point the nuclei lose their normal shape, flatten and move to the periphery of the cell, which has preserved fine-grained formation of the chromatophil substance. For this reason one part of the cell cytoplasm where chromatolis is took place, becomes lucid, and vitrious in some neurons (Fig. 6).

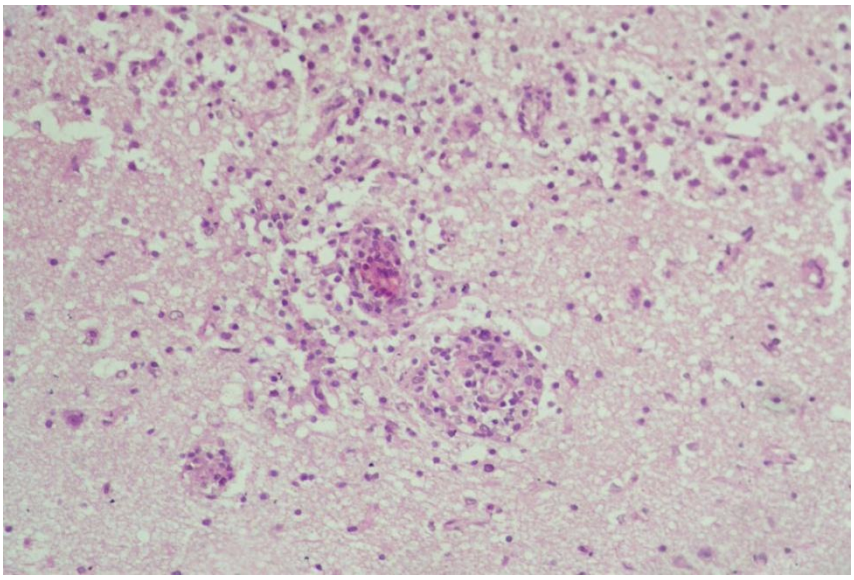

Fig. 6. Perevascularinfiltrates in the brain (Hematoxylin and eosin stains, oc. 10 lens 20)

However, it should be noted that the substance of the brain showed perivascular infiltration in places to form a focal inflammatory infiltrate.

Thusprecapillary walls, venules, and arterioles are often loosened, with endothelium swollen. Cellular infiltrate is composed of lymphocytes, glial cells, and plasma cells. Small, oval, 1-2 micron spores are visible in such areas of the substance or near the cells (Fig. 7).

In some cases, plasmatic vascular*permeability* was discerned. The latter looked thicker and more intensely colored. Around such vessels moderate and intense clusters of cells formed by lymphocytes, histiocytes, and plasma cells were noted (Fig. 8)

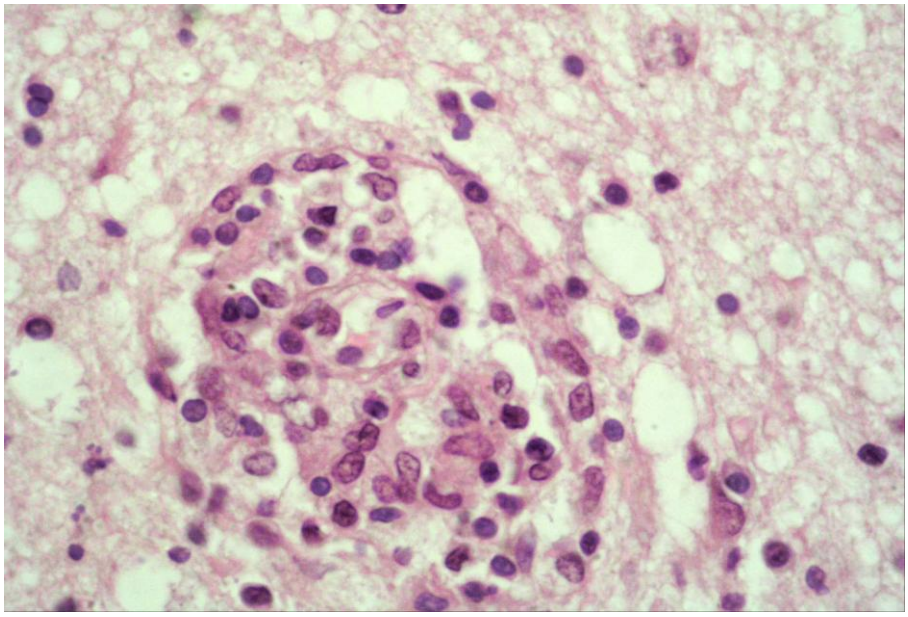

Fig. 7 Brain. The wall of the venulesis loose. Perivascular cellular infiltration (Hematoxylin and eosin stains,oc. 10 lens 100)

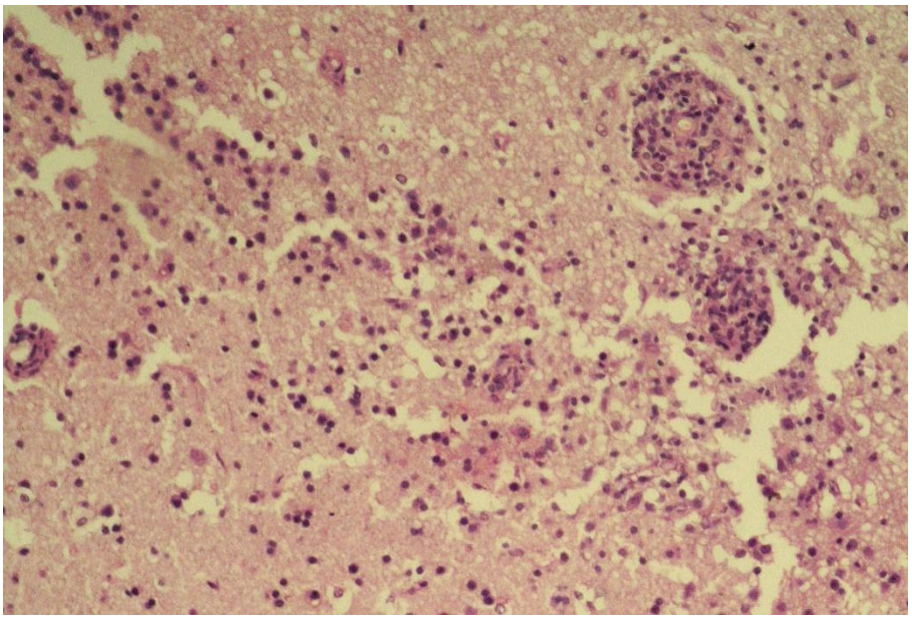

Fig. 8. Brain. Plasmorrhagia of vascular walls. Perivascular sleeves (Hematoxylin and eosin stains, oc. 10 lens 20)

Moreover, an increase in the number of glial elements surrounding small groups of neurons was detected in places (Fig. 9).

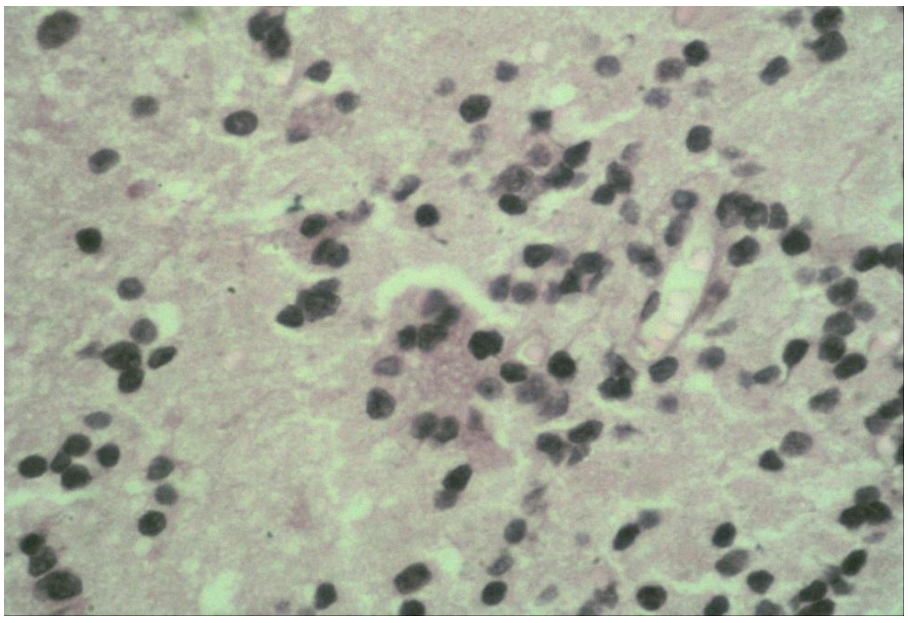

Fig. 9 Brain. Neuronophagia(Hematoxylin and eosin stains, oc. 10 lens 20)

With resolution increased by 1,000 times, one was able to discern the outlines of dead neurons and those in a state of necrobiosis surrounded by glial cells, indicating neuronophagia processes. Scattered small oval clusters of spores were clearly vizualized in these areas of the brain matter.

Consequently, using the results of histological studies it can be argued that hemorrhagic sero-purulent leptomeningit is and focal encephalitis develop in the brain of rabbits caused by reproduction of encephalitozoonosispathogen spores.

Pathomorphological changes in the kidneys of rabbits

In the macroscopic study, both kidneys from the corpses of rabbits were slightly increased in size, bean shaped, of brown color with fine light grayish areas. In section the boundary between the cortex and medulla is not clearly pronounced. Sometimes it was difficult to get an image of the capsule.

Under microscopic examination the severity of microstructural changes in the kidneys of different corpses of rabbits varied. In the kidneys of some corpses of rabbits in which the course of disease was acute, swelling of convoluted tubule epithelium was detected against the background of hyperemia. Vascular congestion of the intermediary zone and glomerular capillary loops was noted. In the proximal tubules the cytoplasm of the cells was granular, sometimes enlightened, unevenly colored, and muddy.

The nuclei were round, with a reduced chromatin content; placed in the center in some cells, in others shifted to the periphery closer to the membrane. The contours of the cells were fuzzy and blurred. The cytoplasm in some cells was enlightened, with nucleilysed. Because of the swelling of *nephrothelium*, the tubular lumen was narrowed in places, the lumen patency disrupted.

An accumulation of eosinophilic, loose protein was observed in the lumen of moderately dilated tubules(Fig. 10).

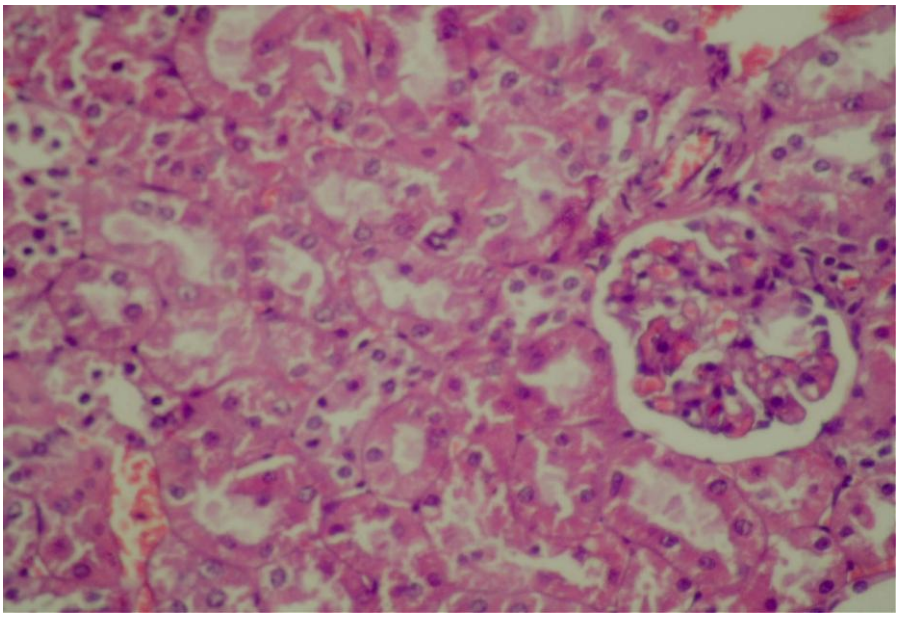

Fig. 10 Granular dystrophy of the epithelium of convoluted tubules. Accumulation of eosinophilic loose protein in the lumen of tubules. (Hematoxylin and eosin stains, oc. 10 lens 40)

Swelling and granulosity of the cytoplasm of nefrocytes was observed in the distal tubules of the nephron and a group of cells in a state of necrosis was detected. An expressed hydropic degeneration of cells is revealed in the epithelium of the straight tubules and ballooning inplaces with rejection of the apical part of the cytoplasm. The contours between cells are feebly marked, sometimes effaced. In some cells the hypochromicnuclei were found near the basement membrane, in others they were lysed. Due to the strong swelling of the epithelium the lumens of the most straight tubules were closed (Fig. 11)

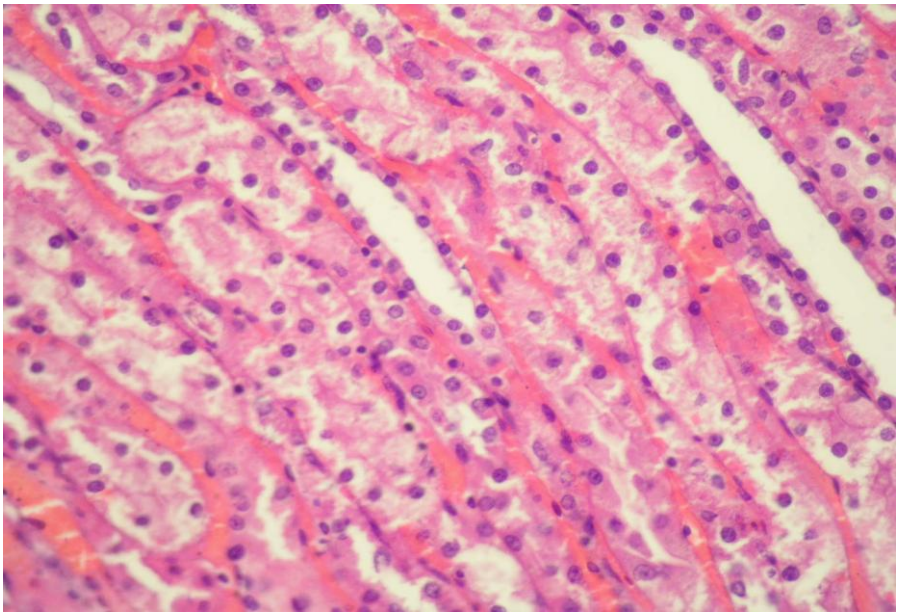

Fig. 11 Hydropic degeneration of the epithelium of the straight tubules (Hematoxylin and eosin stains, oc. 10 lens 40)

Disruption of the tubular structure understandably led to the development of tubular proteinuria. The lumen of the collecting duct loops was slightly enlarged, with epithelium being swollen, weakly basophilic. Shymlanskaya-Bowman Capsule expanded, the glomerular lumen dilated. The basement membrane of the glomerular capillaries was loosened, the endothelium of the vascular plexus swollen, the cytoplasm enlightened, the vascular plexus loops expanded. In addition, the interstices around the glomeruli in some rabbits were sometimes infiltrated by cellular elements, including lymphocytes, histiocytes, and plasma cells, indicating the formation of fine round cellinfiltrates to confirm the development of acute interstitial nephritis (Fig. 12)

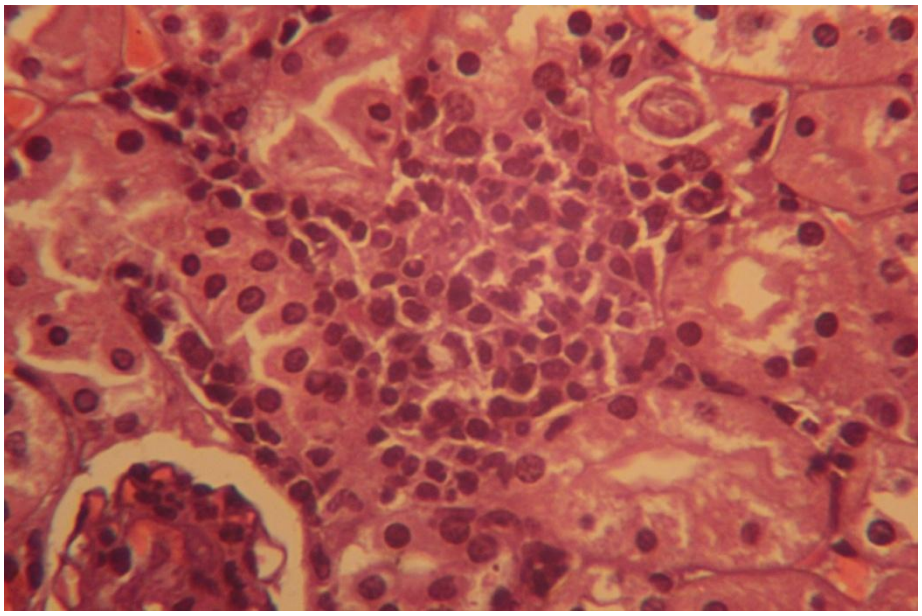

Fig. 12 Round cell infiltration. Swelling of the epithelium of the convoluted tubules
(Hematoxylin and eosin stains, oc. 10 lens 100)

In the kidneys of other rabbits affected by chronic disease the lumen of the proximal tubules was significantly extended. As renal *insufficiency* progressed, epithelicytes of the proximal tubules flattened. Epithelial cells acquired prismatic forms, their contours became fuzzy. The cytoplasm was unevenly colored, muddy, sometimes enlightened. Ther nuclei were round, scantily stained. Significantly pronounced degenerative processes and morphologic alterations of cells were observed in the epitheliocytes of the tubules. The cells were in a state of morphologic alterations; they were characterized by kariolisis and *cytoplasmic constriction.*

Desquamation of tubolocytes was often detected, mainly in the distal region of the nephrons. However, the membranes of the tubules were largely preserved. Shymlanskaya-Bowman capsule was slightly expanded. Aneurysm-like expansion of the capillary loops of the vascular plexus, enlightenment of the cytoplasm of endothelial cells was observed. Red blood cells were revealed in the lumen of the extended capillary loops of the vascular plexus. The detected changes point to an increased permeability of the glomerular filter, which led to the appearance of protein in the tubules (Fig. 13).

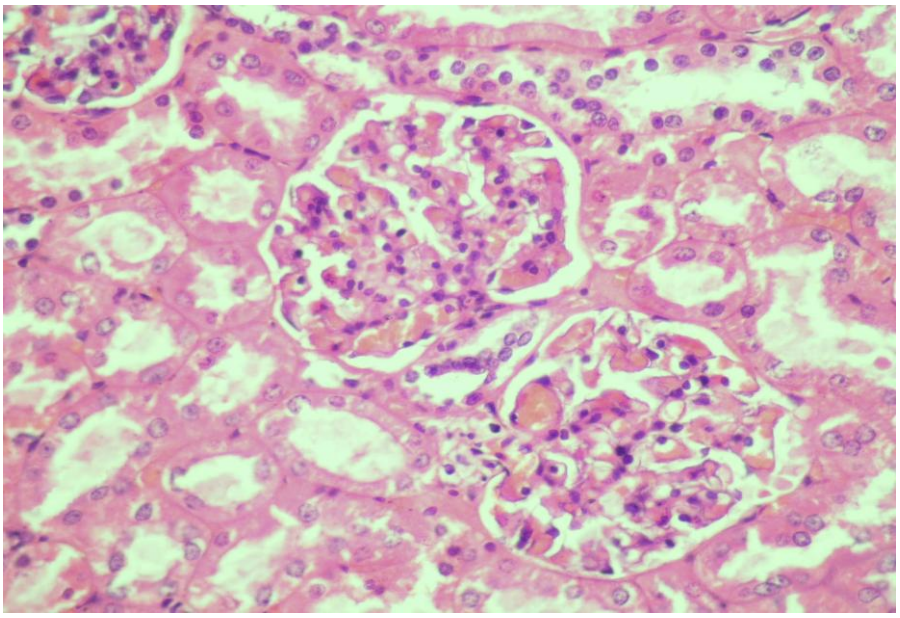

Fig. 13 Expansion of the lumen of the proximal convoluted tubules, focal tubular necrosis (Hematoxylin and eosin stains, oc. 10 lens 40)

Many small arteries showed uneven reduction of the vascular lumen, intimal folding and isolated endothelial vacuolization.

Moreover, thickening of the interstitium between the tubules caused by the growth of connective tissue was sometimes detected. Remnants of lumpy cytoplasm with hyperchromic round nuclei were found in the convoluted tubules condensed near the basement membrane. Pronounced vacuole degeneration in the epithelium of the straight tubules and desquamation of the epithelium was detected. Tubules were clearly affected by lesions from slight focal degeneration to extensive necrosis of epithelial cells, atrophy, and destruction of the tubules. It should be noted that in the slightly enlarged lumen of some straight tubules eosinophilic accumulation of liquid with small oval spores, which are likely to be exreted during urination, was detected. (Fig. 14). At the base of the membranes cells with great hyperchromic nucleus located in the narrow strip of basophilic cytoplasm were sometimes observed.

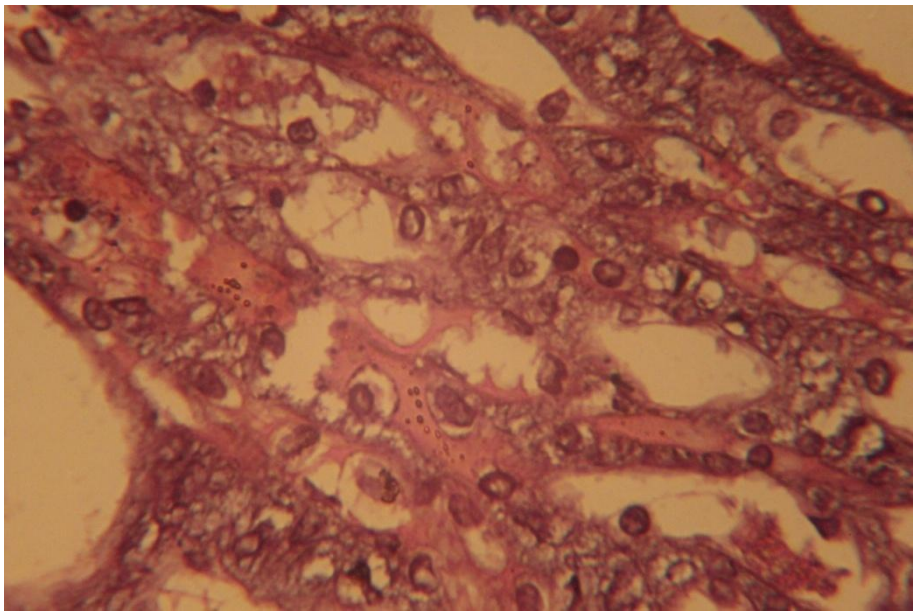

Fig. 14 Straight tubules containing eosinophil fluid and accumulation of small oval spores. Vacuole degeneration and epithelial desquamation (Hematoxylin and eosin stains, oc. 10 lens 40)

Thus, the kidneys of rabbits with acute course of the disease develop complex structural and functional disturbances caused by development of dystrophic-necrobiotic changes in the tubules against the background of hyperemia.

In rabbits with asymptomatic course of the disease, dilatation of the proximal convoluted tubules prevailed in the kidneys together with atrophy of the epithelial cells of the convoluted tubules, focal tubular necrosis, vacuole degeneration and desquamation of epithelium of the straight tubules with moderate growth of connective tissue in the interstices.

The detected changes were caused by the reproduction of encephalitozoonosis pathogen spores. It was confirmed by discovery of small groups of spores, oval in shape, 1-2 microns in size in the lumen of the straight tubules.

Investiogation of liver and lung preparations did not reveal any pathological changes.

DISCUSSION AND CONCLUSIONS

One of the important methods in the diagnosis of encephalitozoonosisin rabbits is pathological and histological studies.

With the death or slaughter of the sick rabbits characteristic pathological-anatomical changes were evident. The bodies were emaciated. Mucous membranes were usually anemic. Typical lesions were observed only in the areas of intense parasitism of pathogens.

With the braincase removed, redness and thickening of the pia mater was observedin the brain. A strong vascular filling of the meninges, the cerebral cortex, and cerebellum was

in evidence. Small foci of softening of the brain tissue were observed in some corpses of rabbits. The brain substance was of pasto seconsistency, with well-defined vascular injection and well defined boundary between the gray and white matter.

Histologically sagittal slices of the brain removed from the carcasses of rabbits showed morphological changes in pia mater, brain stem, less so in cerebellum. Pia mater was affected almost in all the corpses, while inflammatory changes were easily noticed. In other parts of the brain one was able to observe a significant loosening of connective tissue fibers of pia mater and permeation of serous exudate, rich in lymphocytes, erythrocytes, leukocytes, indicating the development of sero-hemorrhagiclepto mening it is. A marked change in the substance of the brain was detected in the areas where pia mater was significantly affected.

Almost all parts of the brain showed hyperemia, pericelar and perivascular edema by optical light. Perivascular infiltration was sometimes observed in the brain substance to form a focal inflammatory infiltrate. The precapillary walls, venules, arterioles were often loosened, endothelium swallen. The cellular infiltrate was composed of lymphocytes, glial cells, and plasma cells. Small, oval, 1-2 micronsin size spores of E. cuniculicould be seen in these areas, in the substance, in or near the cells.

An increase in the number of glial elements was detected in places, which covered neurons in small groups. With an increase by1000 times, it was possible to discern the outlines of dead neurons and those in a state of morphologic alterations, which were surrounded by glial cells. In our view, these changes point to neuronofagia processes. Scattered clusters of small oval spores could be seen in these areas of the brain substance

Thus, serous-hemorrhagiclepto mening it is and focal purulent encephalitis developed in the brain of sick rabbits which was believed to have been caused by the reproduction of encephalitozoonosis pathogen spores.

According to researchers from Turkey and the United States under a microscope the severity of micro structural changes in the kidneys of various animals can vary.

Spores of the pathogen are known to be excreted with urine. For this reason, many research papers are devoted to the changes observed in the urinary system.

In the macroscopic study of kidneys obtained from dead animals with signs of encephalitozoonosis we have established: their size was slightly increased, they were bean shaped, brown with fine light grayish areas. In section the boundary between the cortex and medulla was poorly expressed. The image of the capsule was difficult to obtain.

Under microscopic examination the severity of micro structural changes in the kidneys of various animals varied. In the kidneys of some rabbits in which the disease was acute, one

was able to detect swelling of the epithelium of convoluted tubules against the background of congestion. There was a vascular congestion of the intermediary zone and glomerular capillary loops. The cell cytoplasm in the proximal tubules was granular, sometimes enlightened, unevenly colored, and muddy.

In the kidneys of other rabbits in which the course of the disease was sub acute, proximal tubular lumen was significantly extended. As renal *insufficiency* progressed, epithelicytes of the proximal tubules flattened, epithelial cells acquired prismatic forms, their contours became fuzzy. In rabbits with sub acute course of the disease we observed dilatation of the proximal convoluted tubules in the kidneys, atrophy of epithelial cells of the tubules, focal tubular necrosis, vacuole dystrophy and desquamation of the epithelium of the straight tubules with moderate growth of connective tissue in the interstices.

According to our data in the kidney of rabbits with acute course of the disease a complex of structural and functional disturbances develops caused by development of dystrophic-necrobiotic changes in the tubules against the background of hyperemia.

The detected changes are caused by multiplication of encephalitozoonosis parasite spores.

The obtained data support the findings of Csokai J., and Cox J., who described the brain damage caused by encephalitozoonosis.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

DETECTION AND DIAGNOSIS OF ENCEPHALITOZOONOSIS IN RABBITS UNDER UKRAINE-SPECIFIC CONDITIONS

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KEYWORDS

rabbits, wryneck, Encephalitozoon cuniculi, ELISA, serosanguineous leptomeningitis, focal nonpurulent encephalitis, phacoplastic uveitis

ABSTRACT

For the first time data on detection and spread of encephalitozoonosis in rabbits under Ukraine-specific conditions has been obtained. The disease in rabbits was observed over the year. Encephalitozoonosis in rabbits manifested itself asymptomatically, acutely, and chronically. Asymptomatic course is the most common. In clinical terms encephalitozoonosis manifested itself as severe nervous disorder, defeat of the organs of sight and urinary system.

For the first time in Ukraine, investigation of rabbits afflicted by encephalitozoonosis was conducted with immune ferment method (ELISA) and it was established that the extensiveness of encephalitozoonosis infestation in rabbits reached 34 %. Groups with low (4,97±1,47 absorbance units), medium (16,00±6,14 absorbance units), and high (162,72±58,00 absorbance units) titer and clinically sick animals whose level of antibodies was the highest (391,50±8,10 absorbance units) were singled out from among the rabbits with positive reaction.

Histological investigations revealed sero-hemorrhagic leptomeningitis and focal purulent encephalitis. The detected changes in the organs were caused by reproduction of the parasites in the form of spores.

INTRODUCTION

Encephalitozoonosis is a wide-spread infestation of rabbits. The disease poses danger for productive, fancy, wild, and laboratory populations of rabbits [Keeble E., Shaw D. 2006; Dipineto L. et al. 2008; Valencakova A. et al. 2008; Okewole E. 2008; Cray C. et al., 2009; Ozkan O. et al., 2011, Berezovskyy A. V., Levytska V. A. 2012].

Encephalitozoonosis (syn.: Encephalozoonosis, *Encephalitozoon (nosema) cuniculi*) iszoonotic protozoasis predominantly with cronic or asymptomatic progress. It is caused by small obligate intracellular parasite *Encephalitozoon cuniculi* of *Encephalitozoon* genus which belongs to microsporidia and predominantly affects the central nervoussystem (brain and spinal cord), and – kidneys, liver, spleen, heart, lungs, and eyes of the rabbits. The other two species of this genus – *E. hellem* and *E. intestinalis* are also able to infest mammals [Harcourt-Brown F. 2003; Jordan C. 2005; Valencakova A. 2008; Kunzel F. 2009; Csokai J. 2010; Keeble E. 2011; Sokolova O. et al. 2011].

It is known that encephalitozoonosis afflicts people for whom the source of invasion are

domestic animals, polluted drinking water, or other sick people. However, the main role is played by rabbits as the source of the pathogen invasion [Koudela B. et al. 1999; Deplazes P. et al. 2000; Halanova M. et al. 2003; Snowden F. et al. 2004; Mathis A. et al. 2005; Ditrich O. et al. 2011; Malcekova B. et al. 2011].

In the populations of productive and laboratory rabbits the invasion is widely spread and it affects from34 to 68 % of the local livestock [Ewringmann A., Göbel T. 1999; Harcourt-Brown F., Holloway H. 2003; Ebrecht D., Müller E. 2004, 20. Levytska V. A., Berezovskyy A. V. 2013]. The latest reports argue that the disease started to appear infancy rabbits, which is confirmed by the presence of specific antibodies to *E. cuniculi* in the blood of more than52 % of clinically healthy rabbits [Keeble E., Shaw D. 2006]. The research findings are of great significance as the number of registered instances of disease in people in Europe, North and South America, Africa, and Australia is steadily growing [Mathis A., 2005; Gruber A., 2009]. *E. cuniculi*, is a conditionally pathogenic microorganism posing a threat for people with weakened immune systems including HIV-infected individuals who take immune suppressor preparations, and during organ transplantation[Didier P. et al. 2000; Mathis A. et al. 2005; Flock U. 2010; Sieg J. 2014].

A number of researchers are convinced that E. *cuniculi* is one of the main problems of rabbit breeding. The majority of experts tend to think that the final diagnosis of encephalitozoonosis during the life time is difficult to establish since diagnosis of protozoasis, prevention and treatment are not clarified and not sufficiently developed [Meyer-Breckwoldt A. 1996; Ewringmann A., Göbel T. 1999; Suter C. et al. 2001; Harcourt-Brown F. 2003; Künzel F. et al. 2008; Künzel F., Joachim A. 2010, Levytska V. A., Berezovskyy A. V. 2013].

MATERIALS AND METHODS

The experimental part of the work was performed over 2013–2016 in the laboratory of the Department of Infectious and Parasitic Diseases of the Faculty of Veterinary Medicine at Podillya State Agrarian Engineering University (Kamianets-Podilskyi) and the State Scientific Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Examination (Kyiv). Histological investigation was conducted in the laboratory of Normal and Pathological Morphology and Forensic Veterinary Department of the Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyi (Lviv).

The production test of the research findings was carried out on the farms and the adjoining households in Ukraine.

Rabbits of different breeds and ages, kept under different rearing conditions were examined. Rabbits from 6 weeks to 3 years of age were subjected to research.

In total2563 rabbits were surveyed.

At the first stage of research the spread of encephalitozoonosis in rabbits on the farms of different ownership forms was investigated.

Routine preventive measures were observed during parasitological examination with parasitosis treatment schemes taken into consideration both in the past and current seasons.

At the second stage the effectiveness of laboratory diagnostic methods for encephalitozoonosis in rabbits was determined. For intravital diagnosis 88 samples were examined with enzyme immunoassay (ELISA) blood serums from rabbits. *Encephalitozoon cuniculi* ELISA test-system produced by Medicago AB of Sweden was used. For this test-system antibodies to the encephalitozoonosis pathogen in blood serum are detected in a dilution greater than 1:40. Blood for examination was sampled from clinically healthy rabbits and rabbits with clinical signs of encephalitozoonosis coming from different gender-age groups.

At the third stage of research the influence of encephalitozoonosis pathogen on the organism of rabbits was investigated. Pathological changes in the organs of rabbits, characterized by encephalitozoonosis were determined and microsporidia detected (Csokai, 2009). After the death of sick rabbits, their pathological-anatomical autopsy was performed and tissue samples of the kidneys, liver, lungs and brain (cortex and medulla), hemisphere of the brain and cerebellum) were taken. The tissue samples were fixed in10 % solution of neutral formalin and histological preparations were produced by conventional method, they were stained with hematoxylin-eosin and examined by light microscopy (microscope Leica DM-2500, camera Leica DFC 450c, software Leica Application Suite Version 4.4., oc. 10, lens 100) (Garcia, 2002).

The obtained digital material was processed statistically with MS Excel 2007 computer program with the arithmetic mean definition (M), statistical *error* of the arithmetic mean (m).Probability difference (p) between the arithmetic mean of two variation series was determined by means of the probability measure ment criterion and in accordance with Student's tables (td). The difference between the two values was considered probable with equal probability $p \leq 0,05$.

Investigation was conducted in accordance with the «General ethical principles of experiments on animals» (Ukraine, 2001) and in compliance with the international

requirements of the European Convention «On protection of vertebrate animals used for experimental and other scientific purposes " (Strasbourg, 1985).

RESULTS

Epizootic situation concerning encephalitozoonosis in farm-raised rabbits. According to the research findings, encephalitozoonosis of rabbits was registered on rabbit farms of Ukraine in2014–2016. In rabbits affected by encephalitozoonosis pathogen asymptomatic, acute, and chronic course of disease was observed.

Asymptomatic course of encephalitozoonosisin rabbits is the most common. No clinical signs were observed in the infested rabbits. At the same time, these animals remain parasite carriers. Thus, a serological study showed that in 88 blood samples 30 of them had positive reaction to encephalitozoonosis pathogen, which accounted for 34 %and were indicative of parasitosis.

Acute course of the disease in rabbits manifested itself much less. In some animals the disease developed suddenly with a rapidly increasing symptom complex. The prodromal period of encephalitozoonosisin rabbits varied from several hours to 3weeks, In our opinion, such a state depended on the immune system of their organism . Sometimes, during our research, acute disease course in rabbits was observed under the influence of stress factors. The main factors were transportation, re-grouping and other technological moments including temperature fluctuations of the environment.

Thus clinical symptoms occured among all the age groups. However, the symptoms were sometimes poorly pronounced and depended on the fact which organs were were affected in the first place (table 1).

Table 1. Epizootic situation concerning encephalitozoonosis in rabbits on the research farms

Farms	Number of animals	System of organism	Number of infested animals	Age, sex	Total number of sick animals	EI, %
A.V.Datsiuk's farm	835	Nervous system	8	Young stock (1,5-3 months old)	10	1,19
			2	Adult females		
			-	Adult males		
		Urogenital system	3	Young stock (1,5-3 months old)	4	0,48
			1	Adult females		
			-	Adult males		
		Affection of organs of vision	1	Young stock (1,5-3 months old)	6	0,72
			5	Adult females		
			-	Adult males		
AF Slobodian	1365	Nervous system	11	Young stock (1,5-3 months old)	16	1,17
			4	Adult females		
			1	Adult males		
		Urogenital system	5	Young stock (1,5-3 months old)	7	0,51
			2	Adult females		
			-	Adult males		
		Affection of organs of vision	-	Young stock (1,5-3 months	3	0,22
			3	Adult females		
			-	Adult males		

In the study of 58 sick rabbits over 1–2 weeks, clinical symptoms were observed, with periodic aggravation of symptoms and the appearance of new morbid afflictions of the organism systems (nervous, *urogenital*, and organs of vision). Thus in 37 rabbits neurological

symptoms were observed which most often manifested themselves and amounted to 64 %, among them were found 28 young rabbits aged 1,5–3 months which accounted for 76 %. Defeat of the nervous system mostly manifestated itself as vestibular disorders and in the first place was characterized by distortion of the neck in 37 rabbits (hence the popular name – «wryneck»). A head tilt to the right or left side and circus movements were observed in the sick rabbits (Fig. 1).

Fig. 1. Vestibular disorders in rabbits suffering from encephalitozoonosis

Thus, in the first day of illness, the general state and appetite in rabbits were not affected. Gradually, at 7-20 days, neurological symptoms progressed. Rotational movement around the axis of the body and ataxia appeared in most rabbits. Also, convulsions, paresis of one or two limbs, head tremors, shaking or nodding in the state of repose were noted in 32 rabbits. Nystagmus was observed in three rabbits. The state of those rabbits deteriorated. Later they developped a paresis of the pelvic limbs against which muscle degeneration quickly progressed. The animals ceased to eat food and drink water. Tonic-*clonic seizures* set in. The rabbits fell into a comatose state lasting up to 24–48 hours.32 animals died while in a comatose state.

However, in 5 adult rabbits only a distortion of the neck was observed. Their general state was satisfactory at that. They did not loose appetite, ate and drank well. Over two or three months the above said clinical symptoms of the disease disappeared. Upon recovery female rabbits were capable of mating and bearing offspring.

Morbid affection of the urinary system in the sick rabbits, in the early stages of the disease, took asubclinicalcourse. During this period disorders were insignificant and clinical symptoms were not typical – drowsiness, loss of appetite, which gradually led to anorexia, loss of weight, and dehydration were observed in 11 rabbits. According to our observations,

during the 4-6 week of disease, polyuria, polydipsia and urinary incontinence, leading to azotemia developed in rabbits, which was confirmed by biochemical blood analysis. Such animals were noticeable during clinical survey. They were exhausted, had expressed anemia visible mucous membranes; the genital area was constantly contaminated with urine, which later developed skin maceration. At 6-8 in week 10 rabbits developed acute interstitial nephritis, which manifested itself by a sharp deterioration of general state in animals. Body temperature increased to 40,5° C, polyuria and haematuria were evident. At 5-7 day oliguria or anuria developed, which progressed into acute renal failure. In 10 sick rabbits a state of coma and death were observed, which amounted to 91 % among rabbits with defeat of the urogenital system In one rabbit chronic interstitial nephritis, which later led to chronic renal failure was detected.

Defeat of the organs of vision in rabbits suffering from encephalitozoonosis is an important diagnostic feature. The symptoms manifested themselves within 5-10 days after the defeat of the central nervous system. Thus, in 10 rabbits with defeat of organs of vision, neurological symptoms were also observed in eight rabbits. The skin around the eyes was hyperemic, severe swelling of the eyelids was visible. Iridocyclitis was observed in five rabbits.

In the dynamics inflammation expanded to the choroid of the eye and affected all its parts causing panuveitis in 6 of 8 sick rabbits, which amounted to 75% (typical clinical symptom of encephalitozoonosis).

In two 1.5 month old rabbits, born by different females, reproduction of *E. cuniculi* was observed during fetal infestation in the lens of the eye, as manifested by accumulation of white mass. Thus, inflammation was observed in one eye only.

In nine sick rabbits which survived regression the disease took a chronic course. The clinical symptoms of the acute course observed by us for two to three months disappeared. The completion period of encephalitozoonosis was characterized either by recovery or death of the animals. Out of 58 animals suffering from encephalitozoonosis death occurred in 49, which amounted to 84 %. Death was caused by severe pathological changes developed as a result of action produced by *E. cuniculi* on the organism of the animals and complications due to layers of pathogenic microorganisms.

Enzymatic analysis of encephalitozoonosis. According to the analysis of blood samples from 88 rabbits it was found that 30 blood serums were positive (table 2). Thus, infestation extensiveness observed on Breeder A. V. Datsiuk's rabbit breeding farm determined by ELISA test method in 2014 amounted to 34 %. When analyzing data of the

table it becomes evident that in the groups of animals with ELISA test negative results optical density performance fluctuates within0,66–2,55, privative of antibodies to *E. cuniculi*. While in the groups of animals with ELISA test positive results these values fluctuated within 3,45–400,00, indicating wide variations in immune responses. Based on the antibody titers the animals were divided into five groups.

Table2. Investigation of blood serum in rabbits by ELISA test method for presence of specific antibodies to *E. cuniculi*on Breeder A. V. Datsiuk's rabbit farm

Groups based on ELISA test results	n = 88	Average value of optical density units (M±m)	Boundary value of optical density units (Lim)
Reacting negatively	44	1,89±0,65	0,66–2,55
Seropositive, with low antibody titers	12	4,97±1,47*	3,45–8,50
Seropositive, with medium antibody titers	10	16,00±6,14*	10,50–28,75
Seropositive, with high antibody titers	4	162,72±58,00*	103,88–242,40
Seropositive, with high antibody titers and clinical signs	4	391,50±8,10*	381,00–400,00

Notes: *p<0,001 –compared to reference group

According to the research it was established that clinically healthy rabbits had different antibody titers, from low – 14 %, to high – 9 %. In our opinion, this is indicative of occult parasitosis.

It was also noted that the disease with pronounced neurological signs was accompanied by high antibody titers which approximated the value of400 (absorbance units). these results show transition of the rabbit's organism in from the state of parasitosis to the state of disease with the following clinical manifestations of encephalitozoonosis.

Pathological-anatomical and histological investigation. With the death or slaughter of the sick rabbits, characteristic pathological-anatomical changes became evident. The animal corpses showed exhaustion. Mucous membranes as a rule were anemic. Characteristic morbid affectation was observed only in the areas of intensive parasitism of the pathogen.

As a result of the histological examination of the sagittal slices of the brain of the rabbits' corpses morphological changes were found in the pia mater, brainstem, less so in the cerebellum. Pia mater was affected almost in all the corpses, while inflammatory changes were well visible. Other parts of the brain showed significant loosening of the connective tissue fibers of the pia mater and permeability of serous exudate, rich in lymphocytes, erythrocytes, leukocytes, indicating the development of sero-hemorrhagic leptomeningitis. In the areas of significant affliction the pia mater showed marked changes of the brain substance.

Practically in all the areas of the brain hyperemia, per vascular and pericelaredema was detected by using light-optical techniques. Per vascular infiltration with focal inflammatory infiltrate was observed in places in the brain substance. In these areas, the substance in or near the cells, showed small oval spores of E. cuniculi ranging 1-2 micron in size. Scattered clusters of small oval spores of the parasite could also be seen in these areas of the brain substance. Thus, serous-hemorrhagic leptomeningitis and focal no purulent encephalitis developed in the brain of the sick rabbits which we believe was caused by reproduction of the encephalitozoonosis pathogen spores.

Through microscopic investigation the degree of severity of microstructural changes in the kidneys of different animals varied. In the kidneys of some rabbits, in which the course of the disease was acute, swelling of the epithelium of the convoluted tubule against the background of congestion, and vascular congestion of the intermediary zone and glomerular capillary loops were detected. Due to swelling of the nephrothelium, tubular lumen is narrowed in places, with lumen patency disrupted. In addition, in some rabbits the interstitium around the glomeruli was infiltrated in places with cellular elements including lymphocytes, histiocytes, and plasma cells, i.e. the formation of small globo-cellularinfiltrate was traced indicating the development of acute interstitial nephritis. In the kidneys of other rabbits, where the course of the disease was chronic, the lumen of the proximal tubules was significantly distended. In the epitheliocytes of the tubules significantly pronounced degenerative processes and morphologic alterations of cells were observed. However, interstitial thickening was traced in places between the tubules caused by the extensive growth of the connective tissue. Morbid affection of the tubules is clearly seen to be ranging from minor focal degeneration to extensive necrosis of epithelial cells, atrophy and destruction of the tubules. It should be noted that in the slightly enlarged lumen of some straight tubules eosinophilic accumulation of liquid is viewed, with small oval spores which are likely to be easily evacuated with urine.

Investigation showed no pathological changes in the preparations of the liver and lungs.

DISCUSSION

In recent years there have been significant changes in the structure of the pathogens of invasive and infections diseases of farm animals.

Close international economic relations, intensive migration lead to changes of the habitat of certain parasitic and infectious diseases.

In such circumstances, to effectively combat and prevent their occurrence one has to carefully study the regional characteristics of the pathogen, its distribution and characteristics of the epizootic process. However, in the available reference sources we found no data on encephalitozoonosis in rabbits under Ukraine-specific conditions.

Through investigation of the epizootic situation of encephalitozoonosis in Ukraine, distribution and seasonality of the disease was established. According to the research, encephalitozoonosis in rabbits is recorded both on specialized rabbit farms and in private households.

It was established that infestation in rabbits occurs throughout the calendar year. The data received are confirmed by the investigation conducted in the European countries, indicating a year-round detection of rabbits suffering from encephalitozoonosis. In private households infestation was more often registered in autumn and winter period compared to spring and summer. One may assume that seasonal fluctuation of infestation is linked to the seasonal resistance of the organis mand regrouping of the animals. Our research results are consistent with the data obtained in England, USA, and Austria. It should be noted that morbid affection caused by the encephalitozoonosis pathogen is encountered more frequently in rabbits, aged 1.5 to 3 months and rarely in reproductive animals.

Success in preventing and combating encephalitozoonosis in rabbits depends not only on deep knowledge of the local epizootology, timely diagnosis is also of importance. One of the important methods for diagnosis of the encephalitozoonosis complex isanalysis of clinical signs. In the available literature there are many reports on the clinical course of encephalitozoonosis in rabbits. These reports were contributed at different times by scientists from Switzerland, Germany, Czech Republic, England, and Austria.

When studying the clinical manifestations of encephalitozoonosis it was established that prodrome in rabbits varied from several hours to 3 weeks. The course of invasion took asymptomatic, acute, or chronic forms. Key clinical symptoms were observed among all the age groups. Sometimes the signs were mild and depended on which organs were affected

in the first place. Asymptomatic course of encephalitozoonosisin rabbits was the most common. In rabbits suffering from encephalitozoonosis most likely top be affected are the brain, organs of vision and kidneys, as a result of parasite multiplication in these organs. Clinical symptoms, if they occur, are fairly typical. For making adiagnosis one should take into account the three main symptom complexes. Defeat of the nervous system is an often observed symptom.

Today, according to many scientists, serological investigation is the most important method for diagnosing encephalitozoonosis in animals. According toanalysis of blood samples taken from 88 rabbits, 30 serums were found to be positive. In the groups of animals with negative ELISA test results, absorption indices fluctuated within 0,66-2,55 OOH, indicating the absence of antibodies to *E. cuniculi*. While in the groups of animals with positive ELISA test results these indices fluctuated within 3,4-400,0, indicating wide variations in immune response. According to our research it was established that clinically healthy rabbits had different antibody titers, from low –14 %, to high – 9 %. In our opinion, this indicates occult parasitosis.

It was also noted that the disease with pronounced neurological signs was accompanied by high antibody titers which approximated the 400 (absorbance units)value. These results indicate a transition of the rabbit's organism from the state of parasitosis to disease with the ensuing clinical indications of encephalitozoonosis.

One of the important methods for diagnosing encephalitozoonosis in rabbits remains the method of morph pathological and histological investigation.

Following death or slaughter of the sick rabbits, characteristic pathological anatomical changes showed. Serous-hemorrhagic leptomeningitis and focal no purulent encephalitis developed in the brain of the sick rabbits; we believe it was caused by multiplication of the spores of the encephalitozoonosis pathogen. The obtained data are confirmed by the research conducted by Csokai J., Cox J., who described *morbid* affliction of the brain caused by encephalitozoonosis.

According to scientists from Turkey and USA, microscopic investigations help establish that severity of microstructural changes in the kidneys of different animals may vary. Thus, in the kidneys and brain in rabbits suffering from encephalitozoonosis, structural-functional complex disorders developed as a result of parasite reproduction. The above described disorder is characteristic of encephalitozoonosis and can be used for establishing a final diagnosis.

CONCLUSIONS

The paper summarizes the results of our research on diagnostic methods and epizootology of encephalitozoonosis in rabbits on some farms of Ukraine. Data on the extensiveness of invasion based on the the results of the ELISA test method is submitted. An insight is given into clinical manifestation, pathological-anatomical and histological changes of some organs in the rabbits infested by microsporia spores. Encephalitozoonosis in rabbits was recorded on some farms of Ukraine. This rabbit disease is observed throughout the year. The least infested rabbit population is found in the spring and summer, extensiveness of invasion amounted to 0,63–0,79 %. A gradual increase in the number of the sick animals is noted in autumn with extensiveness of invasion up to0,97 %. Maximum extensiveness of invasion (3,08 %) was registered in the winter season. Encephalitozoonosis was most often observed in young rabbits aged1,5 months, extensiveness of invasion reached 0,65–11,76 %. In adult females extensiveness of invasion amounted to 0,32–6,9 %, in males– 0,16–3,85 % manifesting itself enzootic ally. Encephalitozoonosis in rabbits occurs asymptomatically, acutely and chronically. Asymptomatic course of encephalitozoonosis in rabbits is the most common. There exist three symptom complexes: neurological disorders (tilt of the head, tremor of the head, curvature of the neck, rolling over along the longitudinal axis of the body, pelvic limbs paresis, ataxia, dystaxia, periodic agitation, tetanic seizures); *morbid affliction* of the organs of sight (conjunctivitis, iridocyclitis, paralysis of muscles of the eyeball, panuveitis, phaco plastic uveitis); morbid affection of the urinary system(enuresis, polyuria, polydipsia, acute orchronicinterstitial nephritis).

As a result of ELISA test analysis it is established, that extensiveness of invasion in rabbits suffering from encephalitozoonosis is 34 %. Among the rabbits with positive reaction, groups with low (4,97±1,47 absorbance units), medium (16,00±6,14 absorbance units), and high (162,72±58,00 absorbance units) titer and clinically sick animals whose level of antibodies was the highest (391,50±8,10 absorbance units)were singled out.

Histological investigations have proven that serosanguineous leptomeningitis and focal suppurative encephalitis develop in the brain of rabbits. In the kidneys of rabbits during the acute course of the disease dystrophic-necrobiotic changes are observed in the system of tubules against the backdrop of congestion, during the chronic course - dilatation of the proximal convoluted tubules, atrophy of the epithelial cells of the tubules, focal tubular necrosis and vacuole degeneration and desquamation of the epithelium of the direct tubules

with moderate proliferation of the connective tissue in the interstices. The detected changes in the organs are caused by multiplication of the parasites in the form of spores.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

MICROBIOLOGICAL BASIS OF METHANOGENESIS

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KEYWORDS

ABSTRACT

Methanogenesis was an important process carried out by methanogenic bacteria in Earth's history. Contributed to the origin of natural gas deposits and had a great influence on the composition of Earth's atmosphere. This process continues up to now. The biological formation of methane (CH_4) is the result of a specific type of bacterial energy yielding metabolism. Eucaryotic organisms and blue-green algae have not been reported to produce methane. Methanogens are the only living organisms that produce methane as a way of life. These group of microorganisms belonging to the domain of the Archaea. The archaeobacteria are predominantly terrestrial and aquatic microbes, occurring in anaerobic or hypersaline or hydrothermally and geothermally heated environments, also some occur as symbionts in animal digestive tracts. They consist of aerobes, anaerobes and facultative anaerobes which grow chemmolithoautotrophically, organothrophically or facultatively organothrophically. Archaeobacteria may be mesophiles or thermophiles, with some species growing even above 100ºC. Nevertheless, all methanogenic species share certain unique and unifying physiological properties. Methanogens should no longer be regarded as a mysterious group of poorly studied microbes. They give off methane gas as a byproduct of their metabolism, and are common in sewage treatment plants and hot springs, where the temperature is high and oxygen is absent. Methanogenic archaea convert a few simple compounds such as H_2 + CO_2, formate, methanol, methylamines, and acetate to methane. All methanogenic bacteria can use hydrogen as a sole source of reducing power for methanogenesis (i.e., as an energy source) and for cell carbon synthesis; several species utilize formate, and one species, *Methanosarcina barkeri*, can use methanol. Another metabolic feature shared by several species is the ability to synthesize all cellular carbon from CO_2 while growing at the expense of hydrogen oxidation. However, autotrophy has been difficult to document in some species because of very slow growth in the absence of certain organic compounds. It has been well established that acetate is the major methanogenic precursor in several anaerobic ecosystems. However, acetate has not been demonstrated to serve as the sole electron donor for growth and methanogenesis in pure cultures. Previous isotopic studies of Stadtman and Barker that demonstrated very slow acetate fermentation were performed with "highly purified" cultures of *M. barkeri* and *Methanococcus* species. These cultures contained more than one distinct morphological type. Methanogenesis from all these substrates requires a number of unique coenzymes, some of which are exclusively found in methanogens. H_2-dependent CO_2 reduction proceeds via carrier-bound C1 intermediates which become stepwise reduced to methane. Methane formation from methanol and methylamines involves the disproportionation of the methyl groups. Part of the methyl groups are oxidized to CO_2, and the reducing equivalents thereby gained are subsequently used to reduce other methyl groups to methane. This process involves the same C1 intermediates that are formed during methanogenesis from CO_2. Conversion of acetate to methane and carbon dioxide is preceded by its activation to acetyl-CoA.

INTRODUCTION

Shortly after formation of the Earth’s surface, methane was one of the predominant greenhouse gases, which together with carbon, nitrogen and water vapor affected the composition of the Earth's atmosphere. The beginning of life occurred in this oxygen-deprived atmosphere. Methane has played a very important role in the entire history of life on the Earth, as it was one of the most important gases that caused the greenhouse effect and shaped the Earth’s temperature. In addition to the volcanic origin, methane is produced by a large group of primitive microorganisms called methanogens. Methanogenic bacteria live in extreme environments like hot springs, anaerobic conditions, saline water and by taking part in the breakdown of organic matter they play an important role in the circulation of carbon in nature. The paper presents optimal conditions for methanogenic bacteria, their ecology, systematics, and the processes which are carried out by these oldest bacteria on Earth.

ECOLOGY OF METHANOGENS

Earth is the only planet we know of that can support life. This is an amazing fact, considering that: it is made out of the same matter as other planets in our solar system, was formed at the same time and through the same processes as every other planet, and gets its energy from the sun. Scientists believe that the Earth began its life about 4.6 billion years ago. The Earth formed as cosmic dust lumped together to form larger and larger particles until 150 million years had passed. At about 4.4 billion years, the young Earth had a mass similar to the mass it has today. The continents probably began forming about 4.2 billion years ago as the Earth continued to cool. The cooling also resulted in the release of gases from the lithosphere, much of which formed the Earth's early atmosphere. Most of the Earth's early atmosphere was created in the first one million years after solidification (4.4 billion years ago). Carbon dioxide, nitrogen, and water vapor dominated at that time. Earth's atmosphere was first supplied by the gasses expelled from the massive volcanic eruptions of the Hadean Era. These gases were so poisonous, and the world was so hot, that nothing could survive. As the planet began to cool, its surface solidified as a rocky terrain and the oceans began to form as the water vapor condensed into rain. The key milestones associated with the evolution of Earth's atmosphere over the centuries, are presented in Table 1.

Table 1. Evolution of the Earth's atmosphere (after Pidwirny, 2006)

Name of Stage	Duration of Stage (Billions of Year Ago	Main Constituents of the Atmosphere	Dominant Processes and Features
Early Atmosphere	4.4 to 4.0	H_2O, hydrogen cyanide (HCN), ammonia (NH_3), methane (CH_4), sulfur, iodine, bromine, chlorine, argon	Lighter gases like hydrogen and helium escaped to space. All water was held in the atmosphere as vapor because of high temperatures.
Secondary Atmosphere	4.0 to 3.3	At 4.0 billion H_2O, CO_2, and nitrogen (N) dominant. Cooling of the atmosphere causes precipitation and the development of the oceans. By 3.0 billion CO_2, H_2O, N_2 dominant. O_2 begins to accumulate.	Continued release of gases from the lithosphere. Water vapor clouds common in the lower atmosphere. Chemosynthetic bacteria appear on the Earth some time between 3.9 and 3.5 billion years ago. Life begins to modify the atmosphere.
Living Atmosphere	3.3 to present	N_2 - 78%, O_2 - 21%, Argon - 0.9%, CO_2 - 0.036%	Development, evolution and growth of life increases the quantity of oxygen in the atmosphere from <1% to 21%.500 million years ago concentration of atmospheric oxygen levels off. Humans begin modifying the concentrations of some gases in the atmosphere beginning around the year 1700.

As the Earth continued to cool, the water vapor found in the atmosphere condensed to form the oceans and other fresh water bodies on the continents. Oxygen began accumulating in the atmosphere through photo-dissociation of O_2 from water, and through photosynthesis (life). The emergence of living organisms was extremely important in the creation of atmospheric oxygen and ozone. Without ozone, life could not exist on land because of harmful ultraviolet radiation (Kasting, 2004a).

Most of the oxygen in the atmosphere occurred between 2.1 and 1.5 billion years ago as a direct result of photosynthesis from ocean-based plants like algae. At about 450 million

years ago, there was enough oxygen in the atmosphere to allow for the development of a stratospheric ozone layer that was thick enough to keep terrestrial life protected from ultraviolet radiation. As a result, terrestrial life began its development and expansion at that time. Table 2 describes the timing of the evolutionary development of some of the Earth's dominant forms of life before and after 450 million years before present.

Methane is an important trace gas in the Earth's atmosphere. Even though it only makes up 0.00017% (1.7 parts per million by volume) of the atmosphere, methane traps a significant amount of heat, helping the planet remain warm and habitable.

Table 2. Approximate origin time of the major plant and animal group (after Pidwirny, 2006).

Organism Group	Time of Origin
Marine Invertebrates	570 Million Years Ago
Fish	505 Million Years Ago
Land Plants	438 Million Years Ago
Amphibians	408 Million Years Ago
Reptiles	320 Million Years Ago
Mammals	208 Million Years Ago
Flowering Plants (Angiosperms)	140 Million Years Ago

The amount of methane in the atmosphere is the result of a balance between production on the surface and destruction in the atmosphere. Methane forms when organic matter decomposes in oxygen-poor environments, such as marshes, rice paddies, or the digestive systems of cattle. It also comes from combustion (burning) of carbon-based fuels. Early in the Earth's history - about 3.5 billion years ago there was 1,000 times as much methane in the atmosphere as there is now. The earliest methane was released into the atmosphere by volcanic activity. During this time, the Earth's earliest life appeared. These first, ancient bacteria added to the methane concentration by converting hydrogen and carbon dioxide into methane and water. Oxygen didn't become a major part of the atmosphere until photosynthetic organisms evolved later in Earth's history. With no oxygen, methane stayed in the atmosphere longer and at higher concentrations than it does today. In today's oxygen-rich atmosphere, the carbon in methane is much happier teaming up with the oxygen in hydroxyl radicals to produce CO_2 and carbon monoxide (CO), releasing water vapour in the process. Consequently, methane remains in the atmosphere a mere 10 years and plays

just a bit part in warming the planet. In fact, the gas exists in minuscule concentrations of only about 1.7 ppm. Instead, CO_2 is roughly 220 times and water vapour is 6,000 times as concentrated on the planet's surface (Kasting, 2004b).

In nature, all processes of growth and development of living organisms are related to the use of carbon as one of the most important biogenic elements. The availability of carbon determines the proper functioning of microorganisms, including a fundamental process to life on Earth – photosynthesis. The biological function of carbon lies in the fact that it occurs as a component of proteins, amino acids or ATP. Further, carbon is a part of the chlorophyll molecule, and is essential to the production of carbohydrates (Barabasz, 1991, 1992).

Carbon, after hydrogen, helium and oxygen, is the fourth most common element in the universe. It is present in all living organisms. Together with a variety of organic compounds, this makes it the chemical basis of life. Carbon-containing compounds are the basis of life on Earth, a series of carbon-nitrogen-oxygen provides the energy produced by the sun and other stars. Despite the diversity of carbon compounds, most of these forms are relatively poorly reactive under normal conditions. They do not react with sulfuric acid (VI), hydrochloric acid, chlorine, or bases. At elevated temperatures carbon reacts with oxygen to form carbon oxides, and reduces many metal oxides such as iron oxide, to metal (Lynch and Pole, 1979).

Carbon has the ability to create very long chains containing CC bond. Carbon-carbon bond is very strong and stable. This ability allows to create virtually infinite number of compounds. Number of compounds containing carbon in its structure is greater than all the other compounds that do not contain carbon (except for hydrogen-containing ones, since virtually any organic compound contains an atom of hydrogen). The simplest organic compounds are hydrocarbons, containing only carbon and hydrogen atoms in their structure. Chain length, side chains and functional groups affect their properties.

When the element next to the carbon and hydrogen is oxygen, it creates many groups of biologically important compounds such as sugars, chitins, alcohols, fats, aromatic esters, carotenoids and terpenes. A nitrogen-containing compounds form alkaloids, amino acids, sulfur-containing antibiotics. Phosphorus-containing compounds form DNA, RNA and ATP, the most important energy carriers in the cell.

Apart from organic carbon compounds, there are another important particles such as carbon dioxides, carbon monoxides, carbonic acids, carbonates, and carbides. They are commonly used in alloys with iron (steel and cast iron). Compounds contain usually tetravalent, rarely divalent (such as carbon monoxide and complex compounds) carbon particles. It occurs in various oxidation states ranging from -4 to +4. Methane is one of the

simplest compounds of carbon and hydrogen. In the history of life on Earth it has played a very important role, as a result of the evolution contributing to the occurrence of a specific group of bacteria - methanogens that enabled the occurrence of other bacteria and higher organisms. Methanogenic bacteria play an important role in the carbon cycle (Fig. 1) to produce methane and converting it to more complex compounds that build the cell (Barabasz and Vořišek, 2002; Hanson and Hanson, 1996).

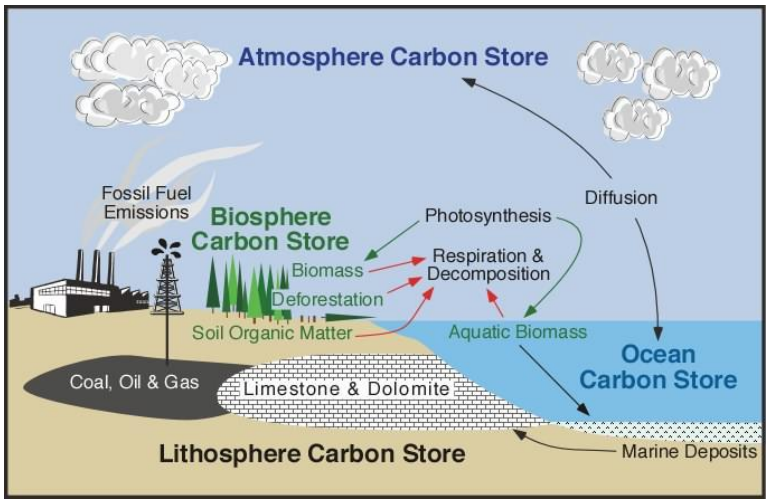

Fig. 1. Carbon cycle in nature (after Pidwirny, 2006).

The whole atmosphere of the early Earth was devoid of oxygen, in consequence of intense volcanic activity it consisted of large amounts of hydrogen. Being unable to combine with oxygen into water, the amount of hydrogen in the atmosphere and oceans was most likely sufficient for the development of methanogens. Taking into account these and other considerations, some researchers believe, that using hydrogen of geological origin by methanogens can form the basis of existence of subsurface microbial systems on Mars and the ice-covered moon of Jupiter – Europa (Kasting, 2004a) . The greenhouse effect caused by the presence of methane was a factor of heat trapping at the surface of the Earth. However, the dominance of methanogens did not last forever. The temperature drop caused by the disappearance of their population probably explains the first and probably the next ice global ages. The abundance of methane in the atmosphere probably caused that pinkish-orange fog enveloped the Earth, similar to the one we see today around the largest of Saturn's moons, Titan (Canfield et al., 2006; Fielding et al., 1988; Garcia et al., 2000).

Methane is the simplest aliphatic hydrocarbon, which is the main component of natural gas and gas found in coalmines or gas generated during anaerobic decomposition of organic material. The main culprits are bacteria that live in wetlands (swamps), rice fields, landfills

and animal remains. Hence the majority of the emissions of methane into the atmosphere is due to agriculture (rice cultivation, cattle breeding) and to a lesser extent due to the extraction and use of natural gas and industrial production. Methane occurs in the free state on the Earth in natural gas fields, where it is mainly obtained. Methane also accompanies coal mining and petroleum industry. It forms a natural gas - an organic fossil fuel, accumulating in the plates of Earth's crust, sometimes at high pressure. The deposits of natural gas occur alone or they are accompanied by deposits of oil or coal. Content of nutrients is variable and depends on the location of extraction, but methane is always the main component constituting over 90% of natural gas. Apart from methane, the gas may consist of small amounts of ethane, propane, butane and other organic compounds and minerals. The current concentration of methane in the atmosphere is about 1745 ppb, but it has doubled over the last 1000 years (Fig. 2).

Methane gas is colorless, odorless and burns with a blue flame. It is lighter than air, and its density at 25°C under pressure of 1 atm is 0.657 $kg \cdot m^{-3}$. The solution of methane solubility in water (at a temperature of 25 °C) is 0.0345 dm^{-3}, and the speed of molecules of CH_4 has a value of 680.9 $m \cdot s^{-1}$ (Fig. 3). It is explosive when mixed with atmospheric air in a volume ratio of 5-15%. Methane in the atmosphere is present in trace amounts, it is chemically active and has the ability to absorb infrared radiation. Its duration in the atmosphere is about 10 years. Methane is an extremely important component of natural gas. A shape of methane molecule in very high magnification resembles a regular tetrahedron. Carbon (C) is denoted as the symbol of sp3 hybridization, by which the orbits forming the bond formed with the other (4) hydrogen atoms. Each bond has the same value, which means that the angles between them are 109°28'. In addition, these bonds have a very weak polarization, which, taking into account the lack of pairs of free electrons, causes that from the chemical point of view methane's longevity is average.

There are many significant sources of CH_4 emissions including natural sources, which include:

- Wetlands,
- Oceans and fresh water reservoirs,
- Volcanic eruptions and cracks in the earth's crust
- Ruminants and termites - fermentation of cellulose in their digestive process.

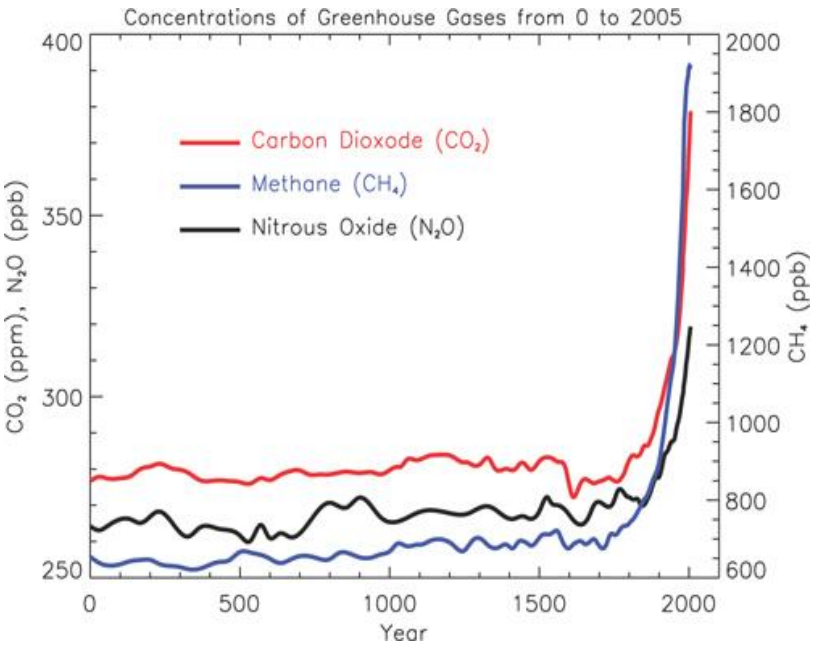

Fig. 2. Concentration of greenhouse gases in atmosphere (after Report IPCC, 2007)

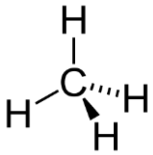

Fig. 3. Structure of methane

Natural sources account for about 30% of global emissions, which is 100-200 Tg per year. In recent years, much attention is paid to methane hydrates, residual at the bottom of the seas and oceans (e.g. $CH_4 \cdot 46\ H_20$). Recently, has been proposed that disturbances associated with the release of methane may be the cause of unexplained sinking of ships and aircraft disappearances in the Bermuda Triangle (Kotelnikova, 2002) . Geological studies confirm the existence of huge quantities of methane hydrates, whose release due to, for example, landslides on the ocean floor or falling meteorite, could cause a global catastrophe. The anthropogenic sources of methane include:

- Extraction of coal, natural gas and crude oil,
- Transport and processing of natural resources,
- Breeding of domestic animals,
- Paddy fields,
- Landfills and sewage treatment plants.

The vast majority of methane occurring in the atmosphere, is produced by anaerobic, decomposing organic matter bacteria called methanogens. These processes occur both in natural ecosystems such as wet meadows, marshes and in anthropogenic ecosystems such as landfills and sewage treatment plants. Most of the anthropogenic emissions have biological origin, and only a small part is caused by incomplete combustion of organic compounds. It is impossible to estimate the share of individual sources in the global methane production. The resulting difficulties have their base mainly in the biochemical nature of methanogenesis, which makes this process determined by many factors and difficult to predict. Methanogenesis processes are unusual natural phenomena. Methanogenesis processes provide opportunities to acquire alternative energy sources in the future. One of the important reactions leading to the formation of methane is methane fermentation, which is a set of molecular reactions that occur with the participation of bacteria. Organic compounds (mostly carbohydrates, proteins, fats and their derivatives) are hydrolyzed and broken down to monomeric compounds such as amino acids, fatty acids monosaccharides and glycerol. This process is called acid genesis (Fig. 4). The resulting monomers are metabolized to acetic acid, and the process is called acetogenesis. CO_2 and H_2 are the by-products of these reactions (Ostafin et al., 2010;Paśmionka et al., 2010).

Polymeric organic substances such as polysaccharides are degraded under anaerobic conditions by intermediates to acetate and H_2, which are substrates for the production of methane. In the last stage the produced acetic acid is transformed into methane by specialized microorganisms (Drake et al., 2008; Ferry, 1992; Hattori, 2008, Hou et al., 1979). The mass balance shows that about 72% CH_4 is produced from acetic acid and the remainder arises from the reduction of CO_2, with the participation of microorganisms. Natural gas is one of the most important sources of methane on Earth, and it was established through biochemical or thermocatalitic processes of organic matter decomposition. In addition, many previous studies of molecular and isotopic composition of natural gas indicate that methane may also be formed in microbial processes. Currently it is assumed, that microbial methane accounts for about 20% in the overall balance of natural gas resources in the world (Kotarba, 1998).

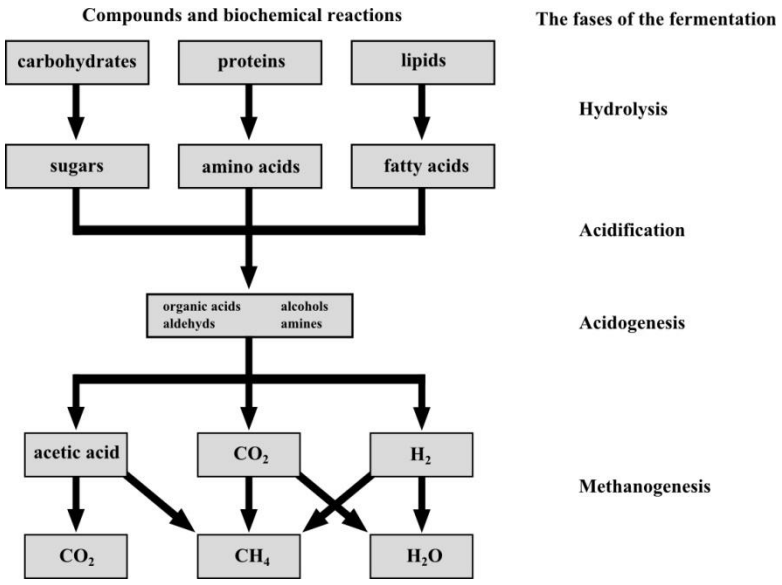

Fig. 4. Biodegradation of organic compounds during fermentation (after Barabasz et al., 2010)

Methane is predominant in natural gas, it is also produced in microbial (bacterial) processes, and in the initial phase of low temperature thermogenic processes (Barabasz et al., 2009). The research developed by Kotarba and colleagues (1987) shown, that the analysis of stable isotopes of carbon and hydrogen in methane can be determined by different environments of deposition of the parent compound from which the gases are formed. Methane gas is dominant, while the amounts of ethane and propane produced during bacterial processes are small. It has been shown that about a thousand molecules of methane are formed per one molecule of ethane.

The Carpathian Foredeep (Poland) is an interesting example where the methane is present as natural gas. Methane accumulated in traps of Miocene Foredeep was produced only during microbial reduction of carbon dioxide, to a depth of about 2,500 m below the sea level in Miocene. It was shown that the greatest intensity of this process reached deep levels between 900 to 1,500 m below the sea bed of the Miocene. It should be noted that for many millions of years there were no clear changes in geothermal conditions in the Carpathian Foredeep. Even the folding and uplift of Oregon and the Carpathian Miocene marine regression probably did not prevent the generation of methane microbial process that can last up to the present day. Microbiological studies have demonstrated the contemporary presence of bacteria in the waters surrounding methane gas deposits in the Miocene. Microbial methane generation and the formation of multilayer traps favored rhythmic and cyclic deposition of silt and sand and a very high rate of sedimentation. Significant reduction in the intensity of this process compared with the sedimentation of Miocene deposits is associated with depletion

of the medium, which is composed of simple organic compounds necessary for the metabolism of methanogenic bacteria (Barabasz et al., 2005, 2009, 2010; Kotarba, 1992; Kotelnikova, 2002).

Biologists have several reasons to suspect that methane-producing microbes were one of the first microorganisms to evolve. They also suggest that methanogens would have filled niches that oxygen producers and sulphate reducers now occupy, giving them a much more prominent biological and climatic role than they have in the modern world (Kasting, 2004a). Methanogens would have thrived in an environment fuelled by volcanic eruptions. Many methanogens feed directly on hydrogen gas (H_2) and CO_2 and belch methane as a waste product, others consume acetate and other compounds that form as organic matter decay in the absence of oxygen. This is why today's methanogens can live only in oxygen-free environment.

On the other hand, geochemists estimate that on the early Earth H_2 reached concentrations of hundreds to thousands of parts per million - that is, until methanogens evolved and converted most of it to methane. Thermodynamic calculations reveal that if other essential nutrients, such as phosphorus and nitrogen, were available, methanogens would have used most of the available H_2 to produce methane.

Methanogenic microbes are thought to be ancient organisms, but it is uncertain when they first appeared on Earth. New results suggest that fluid inclusions found in 3.5-billion-year-old rock samples from the Pilbara craton in Western Australia contain methane that was produced by microbes. This is the earliest evidence for the presence of methanogens, predating previous geochemical evidence by about 700 million years. It represents one of the earliest microbial processes identified in the geological record and suggests that methanogens may have played a role in regulating the climate on the Archaean Earth (Canfield et al., 2006).

Methane-producing bacteria belong to the domain of the oldest known living organisms, Archaea. This is a group of unicellular microorganisms belonging to the prokaryotes. This group was initially named Archaebacteria, but since 1977 it became one of three of the main, separate "domains" of life (together with Bacteria, and Eucaryota) (Woese and Fox, 1977). Methanogens, also known traditionally as methanogenic bacteria, are the Archaea whose main product of respiration is methane. In this type of anaerobic respiration, biologically useful energy is extracted during the transfer of electrons from hydrogen to carbon dioxide. Methanogens require absolute anaerobic conditions for methane production. Their metabolism occurs at temperatures from 0 to 70 °C and some of them may function even at 90 °C, but most of them are killed at higher temperatures. Their metabolic

efficiency increases with the temperature. They have several unique characteristics that distinguish them both from bacteria and eukaryotes, particularly the lipid construction of the cell membrane (structure of isoprenoid moieties, ether linkages at sn-2 and sn-3 positions in glycerophosphate and isoprenoid chains). Originally, Archaea were described as organisms living in extreme environmental conditions. Examples of these environments are highly acidic - or alkaline-water, high saline concentrations or concentrated solutions of other minerals (particularly known for their occurrence in the geysers and hydrothermal vents on the ocean floor), but it turned out that they occur in all habitats and may constitute up to 20% of the total biomass on Earth. Some of these microorganisms may even be found in digestive tracts of various animals, including humans. Physiologically methanogenic bacteria may be aerobes, optional or strict anaerobes. Some of them are mesophilic, others are hyperthermophilic (they can live in temperatures above 100 °C). Their metabolism may be chemolithotrophic and organothrophic.

The microbial group carrying out the methanogenesis is characterized by a dynamic coexistence of different species:

- The first group of bacteria is responsible for hydrolysis of complex particulates by exoenzymes. Monosaccharides, amino acids and fatty acids are formed as a result of these processes. These monomers are then processed by the same or other bacteria to various intermediates, such as hydrogen or acetates and propionates, butyrates, lactates and ethanol.
- The second group of microorganisms - hydrogen-producing acetogenic bacteria convert the previously formed intermediate products of organic substances containing nitrogen to ammonium ions, and hydrogen sulfide ions.
- The third group of microorganisms - methanogenic bacteria produce methane using various components, e.g. acetates.

One of the taxonomic systems of methanogenic bacteria was proposed by the International Committee on Systematics of Prokaryotes in April 2008 (ICSP, 2008) and it was based on the 16S rRNA gene sequence comparisons and genotypic differences.

Detailed studies have shown that Archaea are biochemically and genetically different from the other two domains and have a different structure of the cell membrane, exhibit differences in transcription and translation, and DNA replication.
The genome structure of methanogenic bacteria is similar to the one of eubacteria and consists of a single, circular DNA molecule and frequently occurring plasmids. The nucleotide

sequences encoding proteins exhibit greater sequence homology to eukaryotes than to bacteria. On the other hand, many of their genes are functionally linked operons which is typical of bacteria. Their genes have introns, which were found in genes encoding 23S and 16S rRNA and tRNA. The amino acid sequence (primary structure) of archaeal DNA polymerase is similar to eukaryotic polymerases. Some of them have 3'-5 'exonuclease activity. Polymerase of *Halobacterium halobium* has also the activity of reverse transcriptase. The following enzymes: topoisomerase, gyrase (relieves the tension from the unwinding of the double helix by cutting the strand, and reannealing it once the tension has been released) as well as RM systems (restriction and modification enzymes) have been identified in archaea. The RNA polymerase in archaea forms a complex composed of 14 subunits (compared with 4 subunits of *Escherichia coli.* These subunits show greater homology to the eukaryotic polymerase subunits. Also, as in the case of the eukaryotic cells, the archaeal RNA polymerase is not able to start *in vitro* transcription without having the proper transcription factors (e.g. TFIIB). Their polymerase recognizes a sequence rich in AT pairs from 3225 base pairs upstream the transcription initiation site (analogous to the TATA box sequence in eukaryotes).

It should be noted that detailed studies of Carl Woese team led to a breakthrough in the classification of prokaryotes, for oligonucleotide sequences and specific modification of nucleotides in the 16S-rRNA isolated from methanogenic bacteria were found to be significantly different from both bacterial and eucariotic ones (Woese and Fox,1977; Woese,1981; Woese and Olsen, 1986). It was therefore concluded that metanogens are not Eubacteria and were named Archaebacteria. In subsequent studies other organisms were discovered, mostly the ones isolated from extreme environments, where the 16S-rRNA sequences showed the greatest homology to methanogens. Based on these findings, a new division of the living world into three domains was proposed: Eukaryota, Bacteria and Archaea. The name Archaebacteria was abandoned, as was proved that the Archaea are phylogenetically as distinct from bacteria as from eukaryotes. Universal phylogenetic tree of life, constructed on the basis of differences in 16S-rRNA sequences is shown below (Fig. 5).

Methanogens are microorganisms that produce methane as by-products of metabolism in conditions of very low oxygen concentration. Although they were once classified as Archaebacteria, methanogens are now classified as Archaea, distinct from Bacteria. Methanogens are either rod-shaped or spherical. They do not form a monophyletic group, however the group of methanogens does not consist of a single ancestor and its descendants.

There are over 50 species, all of which belong to the domain Archaea. Methanogens do not require oxygen, and in some cases, cannot even survive in oxygen, though they may be able to tolerate its presence for sustained periods. Methanogens are a very diverse group. They use carbon dioxide or acetate to drive their metabolism, called methanogenesis, along with hydrogen as a reducing agent. Therefore, they have the ecological benefit of removing excess hydrogen and carbon from anaerobic environments. Methanogens that metabolize carbon dioxide are classified as hydrogenotrophic, while those that metabolize acetate are called acetotrophic or aceticlastic (Ferry and Kastead, 2007).

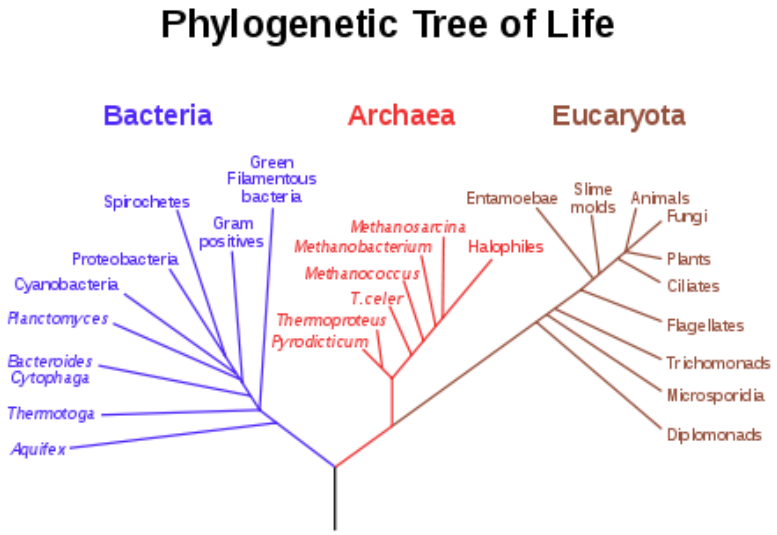

Fig. 5. Phylogenetic tree of life (after Woese et al., 1990).

CLASSIFICATION OF METHANOGENS

The identification of methanogens isolates is very complicated because of their extreme sensitivity to oxygen and the difficulties with isolation and cultivation of pure cultures in a laboratory. Therefore, most probably, we still know only a fraction of methanogens (Whitman et al., 2006).

Phenotypic characteristic is insufficient for identification of methanogens due to their extreme genetic diversity and restricted catabolism. For this reason, analyses of DNA and 16S rRNA sequences are necessary for a definitive assignment of species (Boone and Whitman, 1988; Whitman et al., 2006; Wolfe, 2006).

When analyzing only the last 20 years of advances in systematics of methanogens, it is worth noting, that in 1994 there were 18 genera of methanogenic *Archeobacteria* divided into 3 subgroups (31st group in Bergey's Manual of Determinative Bacteriology) (Holt et al., 1994). Bergey's Manual of Systematic Bacteriology (Garrity et al., 2001) presents

25 genera of methanogens classified in 2 classes, 3 orders and 9 families. On the other hand, 19 genera with over 50 species are mentioned in The Procaryota (Whitman et al., 2006).

The most recent systematics, based partly on Bergey's Manual of Systematic Bacteriology(Garrity et al., 2001) and on identification of 16S rRNA sequences, proposed by International Committee of Systematics of Procaryotes - Subcommittee on the Taxonomy of Methanogens (ICSP, 2008), includes 31 genera (with over 100 species) assigned to 3 classes, 6 orders and 13 different families. Since 2008, several new species of methanogens have been recognized. Moreover, many, still unculturable and unclassified species have been discovered.

The classification of methanogens presented below is based on Bergey's Manual of Systematic Bacteriology(Garrity et al., 2001); the list of taxa covered by the ICSP on the Taxonomy of Methanogens (2008); and recent publications mentioning described and recognized species, which were not mentioned in previous ICSP reports.

Domain *Archae*
Kingdom *Euryarchaeota*
Phylum *Euryarchaeota*

Class *Methanobacteria*

Order *Methanobacteriales*

Family *Methanobacteriaceae*

Genus*Methanobacterium* (type genus) (includes 13 species ICSP: *M. formicicum* (type species), *M. aarhusense,M. alcaliphilum, M. beijingense, M. brytanii,M. congolense, M. espanolae, M. ivanovii, M. oryzae, M. palustre, M. subterraneum, M. thermaggregans, M. uliginosum* and *M. arcticum* (Shcherbakova et al., 2011), *M. kanagiense* (Kitamura et al. 2011), *M. petrolearium* (Mori and Harayama, 2011), *M. ferruginis* (Mori and Harayama, 2011), *M. curvum* (Sun et al., 2001))

Genus *Methanobrevibacter* (includes 13 species ICSP: *M. ruminantium* (type species), *M. acididurans, M. arboriphilus, M. curvatus, M. cuticularis, M. filiformis, M. gottschalkii, M. millerae, M. orali, M. smithii, M. thaueri,M. woesei, M. wolinii* and *M. olleyae* (Rea et al., 2007))

Genus *Methanosphaera* (includes 2 species ICSP: *M. stadtmanae* (type species), *M. cunicculi)*

Genus *Methanothermobacter* (includes 6 species ICSP: *M. thermautotrophicus* (type species) (=*M. thermoalcaliphilum =M. thermoformicicum),M. defluvii,M. marburgensis, M. thermoflexus, M. thermophilus, M. wolfeii* and *M. crinale* (Cheng et al., 2011))

Family *Methanothermaceae*

Genus *Methanothermus* (type genus) (includes 2 species ICSP: *M. fervidus* (type species) and *M. sociabilis*)

Not assigned to any family

Genus *Methanolinea* (includes 1 species ICSP: *Methanolinea tarda* (type species))

Class *Methanococci*

Order *Methanococcales*

Family *Methanococcaceae*

Genus *Methanococcus* (type genus) (includes 4 species ICSP:*M. vannielii* (type species),*M. aeolicus, M. maripaludis* (=*M. deltae*.),*M. voltae*)

Genus *Methanothermococcus* (includes 2 species ICSP: *M. thermolithotrophicus* (type species), *M. okinawensis*)

Family *Methanocaldococcaceae*

Genus *Methanocaldococcus* (type genus) (includes 5 species ICSP: *M. jannaschii* (type species), *M. fervens, M. indicus, M. infernus, M. vulcanius*) and *M. villosus* (Bellack et al., 2011))

Genus *Methanotorris* (includes 2 species ICSP: *M. igneus* (type species), *M. formicicus*)

Order *Methanomicrobiales*

Family *Methanomicrobiaceae*

Genus *Methanomicrobium*(type genus)(includes 1 species ICSP:*M. mobile* (type species))

Genus *Methanoculleus*(includes 6 species ICSP: *M. bourgensis* (type species) (*=M. olentangyi =M. oldenburgensis), M. chikugoensis, M. marisnigri, M. palmolei, M. thermophilus* (*=M. frittonii), M. submarinus*) and *M. receptaculi* (Cheng et al., 2008))

Genus *Methanofollis* (includes 4 species ICSP: *M. tationis* (type species), *M. aquaemaris, M. formosanus, M. liminatans*) and *M. ethanolicus* (Imachi et al., 2009))

Genus*Methanogenium* (includes 4 species ICSP: *M. cariaci* (type species), *M. frigidum, M. marinum,M. organophilum*and *M. boonei* (Kendall et al., 2007))

Genus *Methanolacinia* (includes 1 species ICSP: *M. paynteri* (type species))

Family *Methanoplanaceae*

Genus *Methanoplanus* (type genus)(includes 3 species ICSP: *M. limicola* (type species), *M. endosymbiosus, M. petrolearius*)

Family *Methanocorpusculaceae*

Genus *Methanocorpusculum* (includes 4 species ICSP: *M. parvum* (type species) (*=M. aggregans, M. bavaricum, M. labreanum, M. sinense*)

Genus *Methanocalculus* (The placement of this genus is under study) (includes 4 species ISCP: *M. halotolerans* (type species), *M. chunghsingensis, M. pumilus, M. taiwanensis*)

Family *Methanospirillaceae*

Genus *Methanospirillum* (type genus)(includes 1 species ICSP: *M. hungatei* (type species) and *M. lacunae* (Iino et al., 2010))

Order *Methanosarcinales*

Family *Methanosarcinaceae*

Genus *Methanosarcina* (type genus)(includes 9 species ICSP: *M. barkeri* (type species),*M. acetivorans,M. baltica, M. lacustris, M. mazei* (*=M. fisia), M. semesiae, M. siciliae, M. thermophila, M. vacuolata* and *M. horonobensis* (Shimizu et al., 2011))

Genus *Methanococcoides* (includes 3 species ICSP: *M. methylutens* (type species), *M. alaskense, M. burtonii*)

Genus *Methanohalobium* (includes 1 species ICSP: *M. evestigatum* (type species))

Genus *Methanohalophilus* (includes 3 species ICSP:*M. mahii* (type species),*M. halophilus, M. portucalensis*)

Genus *Methanolobus* (includes 5 species ICSP: *M. tindarius* (type species), *M. bombayensis, M. oregonensis, M. taylorii, M. vulcani*and *M. profundi* (Mochimaru et al. 2009, *M. psychrophilus* (Zhang et al., 2008), *M. zinderi* (Doerfert et al., 2009))

Genus *Methanosalsum* (includes 1 species ICSP: *M. zhilinae* (type species))

Genus *Methanomethylovorans* (includes 2 species ICSP: *M. hollandica* (type species), *M. thermophila*)

Genus *Methanimicrococcus* (includes 1 species ICSP: *M. blatticola* (type species))

Family *Methanosaetaceae*

Genus *Methanosaeta* (type genus) (includes 3 species ICSP: *M. concilii* (type species), *M. harundinacea, M. thermophila* - name is the subject of a pending request for an ICSP opinion)

Family *Methermicoccaceae*

Genus *Methermicoccus* (type genus)(includes 1 species ICSP: *M. shengliensis* (type species))

Order *Methanocellales*

Family *Methanocellaceae*

Genus *Methanocella* (type genus)(includes 1 species ICSP: *M. paludicola* (type species)

Class *Methanopyri*

Order *Methanopyrales*

Family *Methanopyraceae*

Genus *Methanopyrus* (type genus)(includes 1 species ICSP: *M. kandleri* (type species)

Short description of the most numerous methanogenic families

Family *Methanobacteriaceae*:Mostly gram-positive rods – long or short, some of which are cocci, nonomotile, CG content 23-61 mol% (Whitman et al., 2001, 2006; Zhao et al., 1986).

Family *Methanomicrobiaceae:* Gram-negative rods and irregular cocci, motile and nonmotile, CG content 48-52 mol% (Asakawa and Nagaoka 2003; Lai and Chen, 2001;Paynter and Hungate, 1968; Whitman et al., 2001, 2006; Zellner, 1990).

Family *Methanocorpusculaceae:* Gram-negative, small irregular cocci, motile and nonmotile, CG content 39-61 mol% (Lai et al., 2004;Whitman et al., 2001, 2006; Zellner et al., 1987).

Family *Methanosarcinaceae:* Gram-positive or negative, cocci, frequently nonmotile, content CG 36-52 mol% (Boone et al., 1993; Lomans et al., 1999; Maestrojuan et al., 1992; Singh et al., 2005; Sowers and Ferry, 1983; Whitman et al. 2001, 2006).

BIOCHEMISTRY AND PHYSIOLOGY OF METHANOGENS

Methane fermentation is the consequence of a series of metabolic interactions among various groups of microorganisms. In a biogas process, large organic molecules (proteins, sugars and fats) are successively broken down into methane and carbon dioxide, a gas mixture called biogas. A limited number of substrates can be converted to methane, and most species are capable of utilizing only one or two growth substrates. Most of the methane in nature originates from acetate, but some C_1 substrates (carbon-containing compounds that lack carbon-carbon bonds) such as methanol and several different methylamines and methyl sulfides can also be used by some organisms. The first group of microorganisms secretes enzymes which hydrolyze polymeric materials to monomers such as glucose and amino acids, which are subsequently converted to higher volatile fatty acids, H_2 and acetic acid. In the second stage, hydrogen-producing acetogenic bacteria convert the higher volatile fatty acids *e.g.*, propionic and butyric acids to H_2, CO_2, and acetic acid. Finally, the third group, methanogenic bacteria convert H_2, CO_2, and acetate, to CH_4 and CO_2 (Dassonville and Renault, 2002). The various stages of decomposition and the microorganisms that are active at each stage are described below.

Hydrolysis and acid genesis

Hydrolysis is the first stage of the biogas decomposition process. This first stage is very important because large organic molecules are simply too large to be directly absorbed and used by microorganisms as a substrate. Polymeric materials such as lipids, proteins, and carbohydrates are primarily hydrolyzed by extracellular hydrolases excreted by microbes present in Stage 1 (Fig. 4). Hydrolytic enzymes, (lipases, proteases, cellulases, amylases, etc.) hydrolyze their respective polymers into smaller molecules, primarily monomeric units, which are then consumed by microbes (Table 3). In methane fermentation of wastewater containing high concentrations of organic polymers, the hydrolytic activity relevant to each polymer is of paramount significance, in which polymer hydrolysis may become a rate-limiting step for the production of simpler bacterial substrates to be used in subsequent degradation steps. Lipases convert lipids to long-chain fatty acids. The produced long-chain fatty acids are further degraded by p-oxidation to produce acetyl CoA.

Table 3. Selected important groups of hydrolytic enzymes and their functions

Enzymes	Substrate	Breakdown products
Proteinase	Proteins	Amino acids
Cellulase	Cellulose	Cellobiose and glucose
Hemicellulase	Hemicellulose	Sugars, such as glucose, xylose, mannose and arabinose
Amylase	Starch	Glucose
Lipase	Fats	Fatty acids and glycerol
Pectinase	Pectin	Sugar, for example, galactose and arabinose, and polygalactic uronic acid

Proteins are generally hydrolyzed to amino acids by proteases. The produced amino acids are then degraded to fatty acids such as acetate, propionate, and butyrate, and to ammonia.

Polysaccharides such as cellulose, starch, and pectin are hydrolyzed by cellulases, amylases, and pectinases. The majority of microbial cellulases are composed of three types: (a) endo-(3-l,4-glucanases; (b) exo-p-l,4-glucanases; (c) cellobiase or p-glucosidase. These three enzymes act synergistically on cellulose, effectively hydrolyzing its crystal structure to produce glucose. Pectins are degraded by pectinases, including pectinesterases

and depolymerases. Xylans are degraded with □²-endo-xylanase and □²-xylosidase to produce xylose. Polysaccharides such as cellulose, starch, and pectin are hydrolyzed by cellulases, amylases, and pectinases. Hexoses and pentoses are generally converted to C_2 and C_3 intermediates and to reduced electron carriers (e.g., NADH) via common pathways. Most anaerobic bacteria undergo hexose metabolism via the Emden-Meyerhof-Parnas pathway (EMP) which produces pyruvate as an intermediate along with NADH. The pyruvate and NADH are transformed into fermentation endo-products such as lactate, propionate, acetate, and ethanol by other enzymatic activities which vary tremendously with microbial species.

Thus, in hydrolysis and acid genesis (Fig. 4), sugars, amino acids, and fatty acids produced by microbial degradation of biopolymers are successively metabolised by groups of bacteria and are primarily fermented to acetate, propionate, butyrate, lactate, ethanol, carbon dioxide, and hydrogen (Zeikus et al., 1985).

Acetogenesis and dehydrogenation

Although some acetate (20%) and H_2 (4%) is directly produced by acidogenic fermentation of sugars, and amino acids, both products are primarily derived from the acetogenesis and dehydrogenation of higher volatile fatty acids (Fig. 4). Obligate H_2-producing acetogenic bacteria are capable of producing acetate and H_2 from higher fatty acids. Only *Syntrophobacter wolinii*, a propionate decomposer (Boone and Bryant, 1980) and *Sytrophomonos wolfei,* a butyrate decomposer have thus far been isolated due to technical difficulties involved in the isolation of pure strains, since H_2 produced severely inhibits the growth of these strains. The use of co-culture techniques incorporating H_2 consumers such as methanogens and sulfate-reducing bacteria may therefore facilitate elucidation of the biochemical breakdown of fatty acids. Overall breakdown reactions for long-chain fatty acids are presented below:

$CH_3CH_2CH_2COO^- + 2\ H_2O \Rightarrow 2\ CH_3COO^- + 2H_2 + H^+$

$CH_3CH_2CH_2CH_2CH_2COO^- + 4\ H_2O \Rightarrow 3\ CH_3COO^- + 4H_2 + 2H^+$

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2COO^- + 6\ H_2O \Rightarrow 4\ CH_3COO^- + 6H_2 + 3H^+$

$CH_3CH_2CH_2CH_2COO^- + H_2O \Rightarrow CH_3CH_2COO^- + CH_3COO^- + 2\ H_2 + H^+$

$CH_3CH_2CH_2CH_2CH_2CH_2COO^- + 4\ H_2O \Rightarrow CH_3CH_2COO^- + 2\ CH_3COO^- + 4\ H_2 + 2H^+$

However, with a combination of H_2-consuming bacteria, co-culture systems provide favorable conditions for the decomposition of fatty acids to acetate and CH_4 or H_2S.

In addition to the decomposition of long-chain fatty acids, ethanol and lactate are also converted to acetate and H_2 by acetogens and *Clostridium formicoaceticum,* respectively.

Methanogenesis

Methanogenesis is the final stage of the biogas production process. In this stage, methane and carbon dioxide (biogas) are formed by various methane-producing microorganisms called methanogens. The most important substrates for these organisms are hydrogen gas, carbon dioxide, and acetate, which are formed during anaerobic oxidation. But other substrates such as methyl amines, some alcohols, and formats can also be used for the production of methane (Liu and Whitman, 2008).

Energy-Yielding Reactions of Methanogens **(**Boone and Bryant, 1980)

1. $CO_2 + 4\ H_2 => CH_4 + 2H_2O$	-130.7
$HCO_3^- + 4\ H_2 + H^+ => CH_4 + 3\ H_2O$	-135.5
2. $CH_3COO^- + H^+ => H_4 + CO_2$	-37.0
$CH_3COO^- + H_2O => CH_4 + HCO_3^-$	-32.33.
$HCOO^- + H^+ => 0.25\ CH_4 + 0.75\ CO_2 + 0.5\ H_2O$	-36.1
4. $CO + 0.5\ H_2O => 0.25\ CH_4 + 0.75\ CO_2$	-52.75.
$CH_3OH => 0.75\ CH_4 + 0.25\ CO_2 + 0.5\ H_2O$	-79.9
6. $CH_3NH_3^+ + 0.5\ H_2O => 0.75\ CH_4 + 0.25\ CO_2 + NH_4^+$	-57.4
7. $(CH_3)_2NH_2^+ + H_2O => 1.5\ CH_4 + 0.5\ CO_2 + NH_4^+$	-112.2
8. $(CH_3)_2NCH_2CH_3H^+ + H_2O => 1.5\ CH_4 + 0.5\ CO_2 + {}^+H_3NCH_2CH_3$	-105.0
9. $(CH_3)_3NH + 1.5H_2O => 2.25\ CH_4 + 0.75\ CO_2 + NH_4^+$	-170.8

Methanogens can be divided into two groups: H_2/CO_2- and acetate-consumers. Although some of the H_2/CO_2-consumers are capable of utilizing formate, acetate is consumed by a limited number of strains, such as *Methanosarcina* spp. and *Methanothrix* spp. (now, *Methanosaeta),* which are incapable of using formate. Since a large quantity of acetate is produced in the natural environment, *Methanosarcina* and *Methanothrix* play an important role in completion of anaerobic digestion and in accumulating H_2, which inhibits acetogens and methanogens. H_2-consuming methanogens are also important in maintaining low levels of atmospheric H_2. H_2/CO_2-consuming methanogens reduce CO_2 as an electron acceptor via the formyl, methenyl, and methyl levels through association with unusual coenzymes, to finally produce CH_4 (7). Since a small part of the CO_2 is also formed from

carbon derived from the methyl group, it is suspected that the reduced potential produced from the methyl group may reduce CO_2 to CH_4 (Thauer et al., 1989).

Pathways of methanogenesis

Coenzymes and Cofactors

The cofactors involved in methanogenic pathways are shown in Fig. 6. Methanofuran and tetrahydromethanopterin function as one-carbon carriers in the reversible reduction of CO_2 to a methyl group. Molybdopterin guanine dinucleotide was first discovered in a methanoarchaeal formate dehydrogenase and later as a prosthetic group of formylmethanofuran dehydrogenase (IUBMB Enzyme Nomenclature): EC 1.2.99.5), the enzyme catalysing the binding of CO_2 to methanofuran and reduction to formylmethanofuran. The cofactors and coenzymes involved in methanogenic pathways are presented in Fig. 7, some of them can be found outside the Archaea domain.Methanofuran (MF) (Leigh et al., 1984**)** and tetrahydromethanopterin (THMPT) (Keltjens et al., 1983) function as one-carbon carriers, the latter coenzyme also functioning in methylotrophic microbes from the bacteria (Christoserdova et al., 1998).

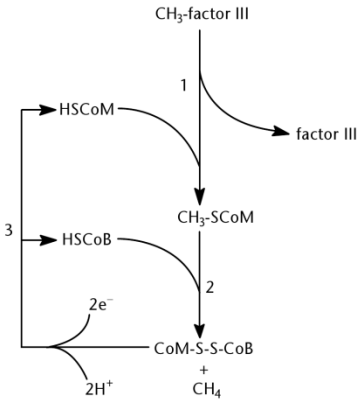

Fig. 6. Reactions and enzymes common to all methanogenic pathways. Step 1, methyltransferase; step 2, methyl–coenzyme M methyltransferase; step 3, heterodisulfide reductase (Ferry, 2002)

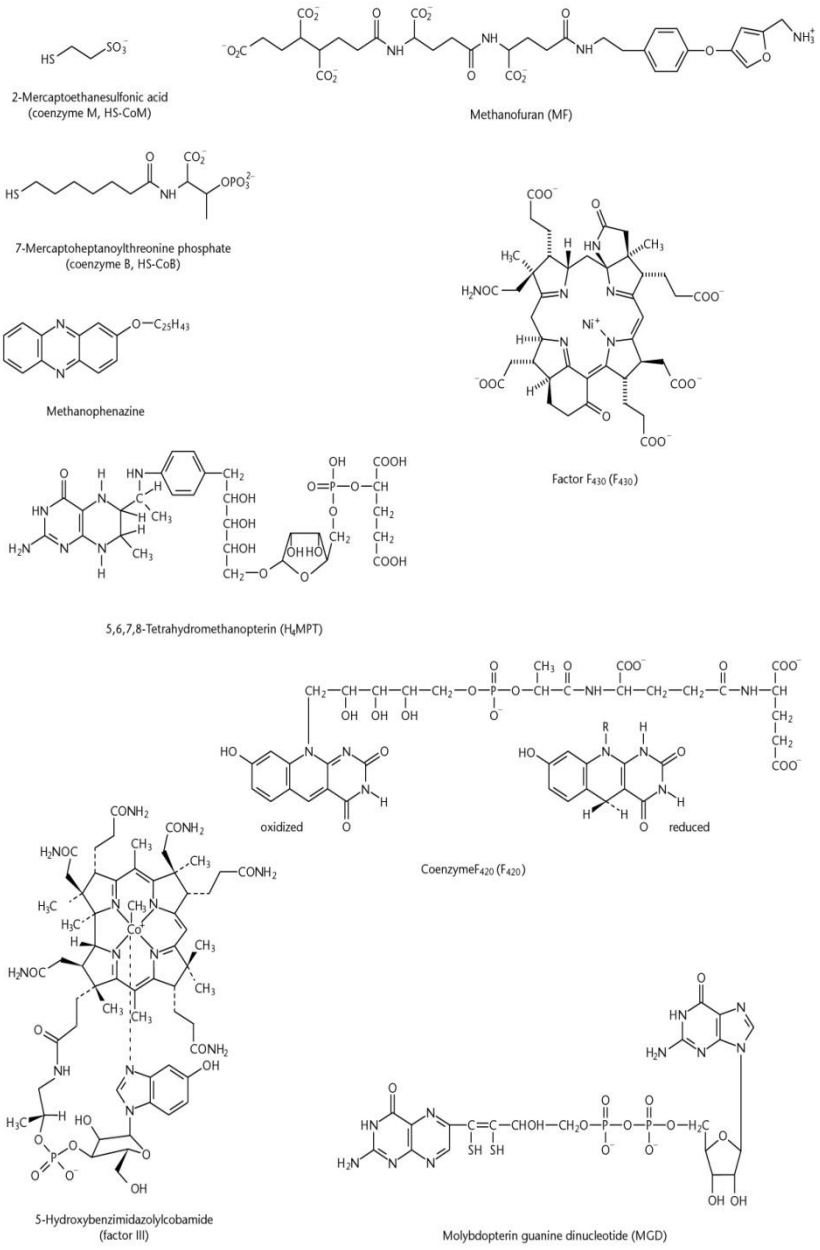

Fig. 7. Cofactors utilized in methanogenic pathways (Ferry, 2002)

Molybdopterin guanine dinucleotide, common in enzymes was first discovered in Archaea as a cofactor of formate dehydrogenase (Karrasch et al., 1990). Coenzyme F_{420}, a dezaflavin derivative (Eirich et al., 1978) is an obligate two-electron carrier accepting or donating a hydride ion. Methanophenazine (MP) (1) is a 2-hydroxyphenazine derivative connected by ether linkage to a polyisoprenoid side chain that functions as a membrane electron carrier (Abken et al. 1998). Factor III is a cofactor of several methyltransferases and has a structure similar to vitamin B_{12}, a major exception being that factor III contains a 5-hydroxybenzimidazolyl base (Scherer et al., 1984). Factor III functions

in methyltransferase by accepting a methyl group as the upper axial ligand to the cobalt. Coenzyme M is the smallest known cofactor (Taylor and Wolfe, 1974). The methylated form of (CH_3-S-CoM) is the substrate for the methylreductase, which catalyzes the reductive demethylation of CH_3-S-CoM to methane in all methanogenic pathways. Coenzyme B (COB-SH) is the second substrate for methylreductase, providing electrons for the reaction (Noll et al., 1986). Cofactor F_{430} (F_{430}), the prosthetic group of methylreductase, was the first nickel-containing cofactor to be described (Diekert et al., 1980; Whitman and Wolfe, 1980). Considerable progress has been made on the biosynthesis of several of the above-mentioned cofactors, including the characterization of enzymes in the biosynthetic pathways (Graham et al., 2001; Graupner and White, 2001; Graupner et al., 2002a, b; Howell et al., 2000; Rasche and White, 1998; Scott and Rasche, 2002; Shima et al., 2002; Solow and White, 1997a, b; White, 1988a, b, 1989a, b, 1990, 1992, 1994, 1996, 1998).

Methanogenesis from CO_2

The process of methanogenesis from CO_2 starts with activation of the CO_2 by the unique cofactor methanofuran, resulting in the formation of formyl-methanofuran is given in Fig. 8. (Escalante-Semerena et al., 1984; Wolfe, 1985). The formyl group is then transferred to another cofactor, tetrahydromethanopterin. A succession of transformations, catalyzed by methenyl-H_4MPT cyclohydrolase, H_2-forming methylene-H_4MPT dehydrogenase, and finally F_{420}-dependent methylene-H_4MPT reductase, which depends on the methanogenic cofactor cofactor F_{420}, results in the formation of 5-methyl-tetrahydromethanopterin (Leigh et al., 1985). The methyl group is then transferred by methyl-H_4MPT: coenzyme M methyltransferase to coenzyme M, resulting in methyl-CoM, which is then disproportionated into methane and CO_2, as described above (Gunsalus and Wolfe, 1977). Since only one in four methyl-CoM molecules is oxidized to CO_2, providing the six electrons that are required for the reduction of the other three methyl-CoM molecules to methane, the next reaction is conversion of CO_2 to methane (Blaylock and Stadtman, 1966; Donnelly et al., 1985; Romesser and Wolfe, 1982;Shapiro and Wolfe, 1980; Wasserman et al., 1983).

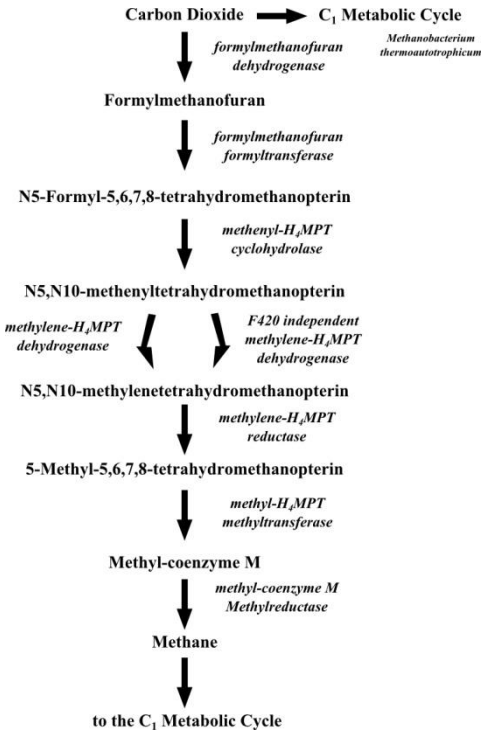

Fig. 8.Methanogenesis from CO_2.

Methanogenesis from formate

Many methanogens are capable of utilizing formate in addition to H_2 + CO_2. Formate utilization starts with its oxidation to carbon dioxide which enters the carbon dioxide reduction pathway outlined above (Sparling and Daniels, 1990). The reducing equivalents generated in this reaction are subsequently utilized to reduce carbon dioxide to methane. The electron-transfer route from formate to the various intermediates is not completely clear yet. Molecular hydrogen, although produced in small amounts, does not appear to be an obligate intermediate in methane formation from formate (Schauer and Ferry,1980, 1986).Since formate dehydrogenases of *M. formicicum* and *M. vannielii* couple the oxidation of formate with the reduction of F_{420}, it is plausible to assume that the reduced F_{420} is directly used in the methylene-H_4MPT dehydrogenase and in the methylene-H_4MPT reductase reactions. Whether the route of electron flow from formate to formyl MF dehydrogenase and to heterodisulfide reductase involves F_{420} as well, is not yet known. The key enzyme of the formate metabolism is formate dehydrogenase. It was purified from *M. formicicum* and *M. vannielii* (Barber et al., 1983; Jones and Stadtman, 1981).The *M. formicicum* enzyme contains molybdenum, zinc, iron, acidlabile sulfur, and one mol of FAD per mol of enzyme. The molybdenum is part of a molybdopterin cofactor whose spectral properties indicate a structural similarity to the molybdopterin cofactor present in xanthine oxidase (Johnson et al., 1991; Schauer and Ferry, 1986).

Methanogenesis from acetate

Most of the methane produced in nature derives from the methyl group of acetate:

$CH_3CO_2^- + H2O \Rightarrow CH_4 + HCO_3^-$

Only two genera of methanogenic acetotrophs are known: the *Methanosarcina* and the *Methanosaeta*. They can obtain energy by conversion of acetate to methane and carbon dioxide. The pathway involves cleavage of the acetyl group of acetyl coenzyme A (acetyl-CoA). The methyl group is transferred to coenzyme M through postulated methyl-corrinoid and methyl-tetrahydromethanopterin intermediates (Thauer et al., 1989);oxidation of the carbonyl group to CO_2 supplies the electron pair required for reductive demethylation of CH_3-S-coenzyme M to CH_4 (Bott et al., 1986; Nelson and Ferry, 1984), the final step in the pathway. All acetotrophic methanogens contain high amounts of carbon monoxide dehydrogenase (CODH), which is hypothesized to catalyze the cleavage of acetyl-CoA, analogous to the reverse reaction catalyzed by the CODH from acetogenic organisms (Krzycki et al., 1985; Terlesky et al., 1987).Acetate is phosphorylated and activated to acetyl-CoA, which is then processed by a large enzyme complex, known as the acetyl-CoA decarbonylase/synthase complex (Fig. 9).

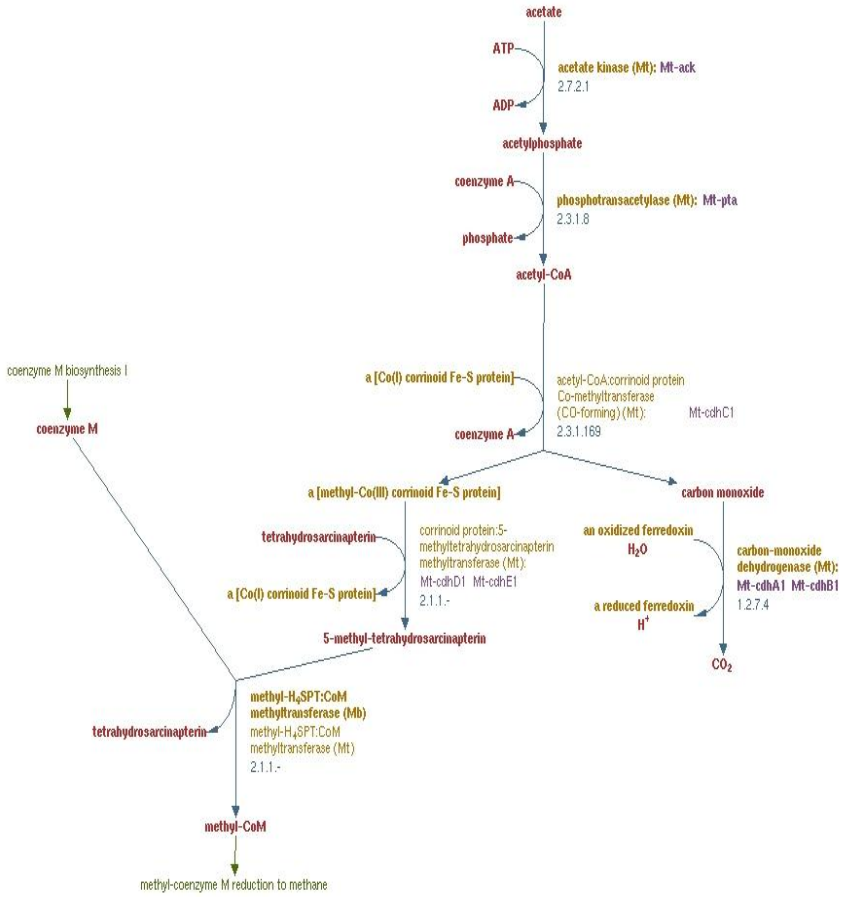

Fig. 9. Methanogenesis from acetate (BioCyc. Database Collection, 2012a).

This enzyme complex cleaves the C-C and C-S bonds in the acetyl moiety of acetyl-CoA, oxidizes the carbonyl group to CO_2 and transfers the methyl group to tetrahydrosarcinapterin. The enzyme can also catalyze the synthesis of acetyl-CoA, a reaction that is not used in methanogenesis, but for autotrophic growth on one-carbon substrates (Abbanat and Ferry, 1990; Ferry, 1992; Maupin-Furlow and Ferry, 1996; Thauer et al., 1989; Terlesky et al., 1986).

The large (~2000 kDa) complex is composed of three enzyme components and five different subunits, with a structure of $(\alpha_2\varepsilon_2)_4(\beta)_8(\gamma\delta)_8$. The specific components of the complex catalyze several partial reactions (Grahame and DeMoll, 1996). CO dehydrogenase activity (EC 1.2.7.4) is catalyzed by the αε component (Balbo and Oliveira, 2003; Gong et al., 2008), acetyl-CoA: corrinoid protein Co-methyltransferas activity, along with CO production (EC 2.3.1.169) is catalyzed by the β subunit, and the rapid, reversible transfer of a methyl group between the γδ component's corrinoid cofactor and tetrahydrosarcinapterin (EC 2.1.1.-) is catalyzed by the γδ component. The methyl group, which has been transferred to tetrahydrosarcinapterin, is then transferred to coenzyme M producing methyl-CoM, which

is reductively demethylated to methane with electrons from coenzyme M and coenzyme B (see methyl-coenzyme M reduction to methane). The resulting heterodisulfides of coenzyme M and coenzyme B are then reduced to their sulfhydryl forms (see coenzyme B/coenzyme M regeneration) (Ferry, 1992, 1997).

Methanogenesis from methanol and methylamines

Methane production from methanol was first observed in *Methanosarcina* by Stadtman and Barker (1951). *M. barkeri* is a methanogenicarcheon that can grow on acetate and various one-carbon compounds such as CO_2, methylamine, and carbon monoxide, as well as methanol, as a sole energy source. Growth on methanol has been reported both in the presence and absence of H_2; when hydrogen is not available, the reduction equivalents needed in methanogenesis are derived from disproportion of the methanol, leading to the oxidation of quarter of the methanol to CO_2. The conversion of methanol and methylamines to methane and CO_2 (reaction [3]) is a dismutation event in which the methyl group of one substrate molecule is oxidized to CO_2 (reaction [1]) providing six electrons for reduction of the methyl groups of three substrate molecules to methane (reaction [2]):

$R\text{-}CH_3 + 2H_2O \Rightarrow RH + CO_2 + 6e^- + 6H^+$ [1]
$3R\text{-}CH_3 + 6e^- + 6H^+ \Rightarrow 3CH_4 + 3RH$[2]
$4R\text{-}CH_3 + 2H_2O \Rightarrow 4RH + 3CH_4 + CO_2$[3]

(where R= –SH, –OH, $–NH_2$, $–NHCH_3$, $–N(CH_3)_2$ or- $N(CH_3)_3^+$).

Reaction [1] is accomplished by transfer of the methyl group to H_4SPT and oxidation to carbon dioxide by a reversal of reactions in the CO_2 reduction pathway. Methyl transfer to H_4SPT has not been investigated; however, methyl group reduction is known to begin with transfer to CoM catalyzed by methyltransferase systems specific for each substrate (Fig. 10.). Reaction [2] involves reduction to methane catalyzed by the methyl–CoM reductase and heterodisulfide reductase common to all methanogenic pathways. The methanol: coenzyme M methyltransferase system from *M.barkeri* contains two components which catalyze sequential reactions (reactions [4] and [5]) leading to an overall transfer of the methyl group of methanol to HS–CoM (reaction [6]).

$CH_3OH + Co^+ [MtaBC] + H^+ => Co^{3+} –CH_3[MtaBC] + H_2O$ [4]

$HSCoM + Co^{3+} –CH_3[MtaBC] => Co^+[MtaBC] + CH_3\text{-}SCoM + 1H^+$ [5]

$CH_3OH + HSCoM => CH_3\text{-}SCoM + H_2O$ [6]

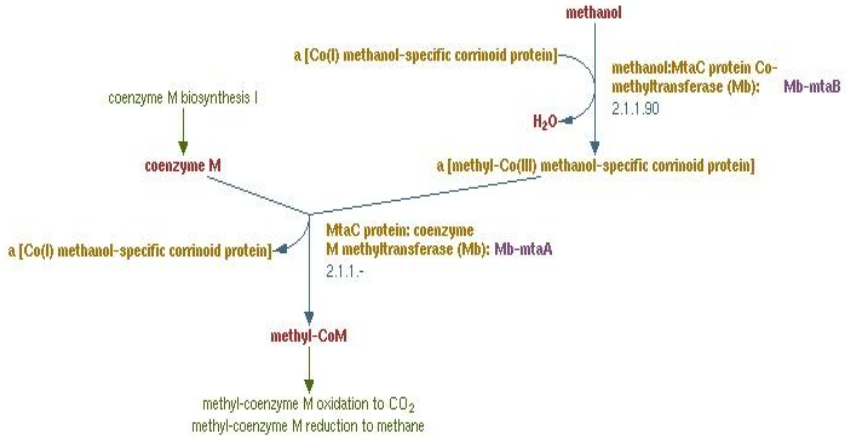

Fig. 10. Methanogenesis from methanol (BioCyc. Collection, 2012b).

The methanol: corrinoid methyltransferase component contains the subunits MtaB plus MtaC and autocatalyses methylation of its factor III prosthetic group in MtaC (reaction [4]). The methyl-corrinoid: HSCoM methyltransferase component catalyses reaction [5] and contains only one subunit (MtaA) with no prosthetic groups. The methanol: corrinoid methyltransferase is purified with the cobalt atom of factor III in the inactive Co^{2-} redox state that requires reactivation by reduction to Co^-, a result consistent with a mechanism in which Co^- is a super nucleophile attacking activated methanol (Sauer et al., 1997). MtaB contains one zinc molecule and its activity depends on this metal; thus, it is proposed that zinc functions as a Lewis acid in the activation of methanol. MtaA also contains zinc and it is postulated that zinc binds to and activates HSCoM for nucleophilic attack on the methyl group of methyl–factor III. The membrane-bound electron transport chain that delivers electrons to the heterodisulfide reductase, and generates a proton gradient for driving ATP synthesis, is fairly well defined. Oxidation of the substrate involves the reduction of F_{420} except the final CO_2-releasing step, for which the electron acceptor is unknown. A F_{420} H_2 dehydrogenase oxidizes $F_{420}H_2$ and transfers electrons to methanophenazine and finally to a b-type cytochrome that donates electrons directly to the heterodisulfide reductase. Additional electron acceptors may be required to couple electron transfer from methanophenazine to the b-type cytochrome. The dismutation of methylamines follows the same general principles as for methanol dismutation. A two-component system is involved

where the first component contains two subunits, one that demethylates the substrate transferring it to the second subunit-containing factor III (Burke and Krzycki, 1997). The first component is specific for monomethylamine, dimethylamine and trimethylamine. However, the second component that transfers the methyl group from factor III to CoM is interchangeable with all three systems. The membrane-bound electron transport chain that delivers electrons to the heterodisulfide reductase, and generates a proton gradient for driving ATP synthesis, is not as well defined as it is for the dismutation of methanol Fig. 11).

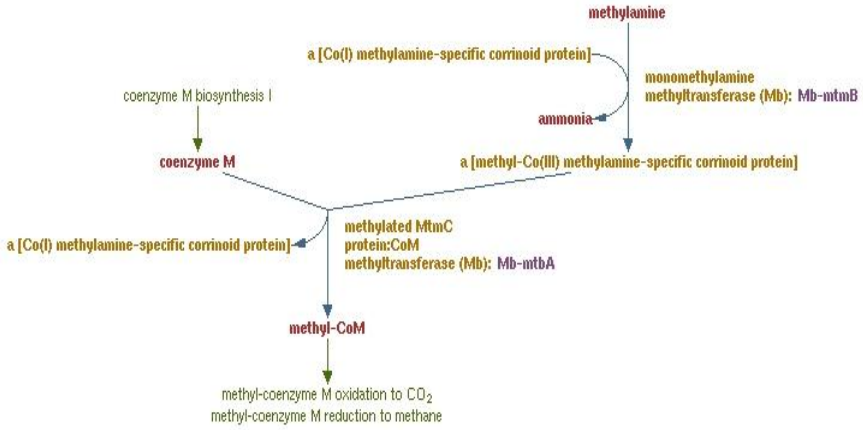

Fig. 11. Methanogenesis from methylamine (BioCyc. Collection, 2012c).

Autotrophy in Methanogens

Methanogen autotrophy is a newly recognized type, in which the key intermediate of carbon fixation is acetyl CoA and the first known CO_2 fixation product is formyl-MFR (Leigh et al., 1985; Ruhlemann, et al. 1985). Formation of acetyl CoA in methanogens is clearly interlinked with catabolism: CO_2 is both the primary electron acceptor and precursor to all anabolites. In *Methanobacterium* spp about 95% of the carbon dioxide fixed is used for energy production, and 5% is used for biosynthesis (Stupperich and Fuchs, 1984a; Thauer and Morris, 1984; Zeikus et al., 1985).The *de novo* synthesis of acetate involves anabolic and catabolic intermediates and cofactors unique to methanogens. The converging pathways of C_1 and C_2 of acetate are clearly seen (Stupperich and Fuchs, 1984b).CODH is centrally involved in acetate synthesis as shown by *in vivo* experiments. Carbon monoxide is incorporated into acetate, and the carboxyl of acetate exchanges with free CO (Stupperich and Fuchs, 1984a, b; Stupperich et al., 1983).A small amount of CO is also synthesized by autotrophic methanogens (Eikmanns et al., 1985).Methanogens which are incapable of autotrophic growth lack CODH. This finding explains their dependence on preformed acetate (Bott

et al., 1985).The methyl carbon of acetate is derived from the H_4MPT-dependent series of reactions that lead to methane (Lange and Fuchs, 1985).The direct involvement of H_4MPT in acetogenesis *in vitro* was demonstrated. The role of cobamides is also significant (Kenealy and Zeikus, 1981).Details concerning the transfer of the methyl moiety and the identity of the other individual components remain unknown. The total synthesis of acetate by methanogens is strikingly analogous to the total synthesis of acetate by certain eubacteria: *Clostridium thermoaceticum, Eubacterium limosum, Butyribacterium methylotrophicum, Acetobacterium woodii,* and *Desulfovibrio baarsii* (Eden and Fuchs, 1982; Ljungdahl and Wood, 1982; Pezacka and Wood, 1986; Ragsdale et al., 1983; Ragsdale and Wood, 1985; Thauer and Morris, 1984; Walker et al., 1984; Zeikus et al., 1985). Many of these organisms are capable of autotrophic growth on hydrogen and carbon dioxide (or carbon monoxide alone), and all of them carry out the synthesis of acetate from CO_2 (Ljungdahl and Wood, 1982; Ragsdale and Wood, 1985). Enzymes of each of the steps shown have been purified to homogeneity and studied in detail. The methyl and carboxyl carbons are derived from separate, converging pathways. CO_2 is reduced via formate dehydrogenase, and then bound to H_4-folate by the ATP-dependent formyltetrahydrofolate synthetase. The carbon is then reduced in a stepwise series of reductions to the methyl level in CH_3-H_4-folate. The source of the carboxyl carbon is CO_2 or pyruvate. However, carbon monoxide will substitute for pyruvate as a carbonyl donor. The terminal step of acetate synthesis, in which the methyl moiety is condensed with a carbonyl equivalent, occurs through the nickel-containing CODH (Ragsdale et al., 1983; Ragsdale and Wood, 1985). This is a new concept, since for some time it was believed that a cobamide enzyme-mediated methyl transfer, and not CODH-mediated carbonyl transfer, was the central step in acetogenesis. Modifications of the pathway unique to methanogens are the ATP-independent activation of CO_2 by the methylreductase and formation of formyl-MFR. The sparing of ATP by utilizing a portion of the free energy of the methylreductase reaction is a beneficial adaptation. Thus, cobamide and CODH-dependent acetate syntheses occur in bacteria of quite diverse groupings: methanogens, acetogens, clostridia, and *Desulfovibrio* spp. This pathway may be used catabolically or anabolically, depending upon the physiological circumstances and the organism (Tanner et al., 1981). Despite variations, such as the replacement of folate with methanopterin, and the differences in the first steps of the pathway (requiring ATP in the eubacterial case and methyl coenzyme M reduction in the methanogen case) and other twists that may occur in the diverse eubacterial acetate synthesizers, the central mechanism of acetogenic autotrophy is conserved. Nickel-containing

CODH seems to be the key enzyme which forms acetyl-CoA from methyl and carboxyl equivalents from divergent pathways.

CONCLUSIONS

Methanogenes played an important role in the history of the Earth, in shaping the composition of the atmosphere and the creation of life. As one of the first organisms on Earth, being the oldest known bacteria involved in numerous processes associated with the formation of organic matter and its accumulation. They have created such deposits of natural gas and possibly oil. Their activity in the prehistory of the Earth was very high, but with increasing oxygen content in the atmosphere and the importance of their participation decreased, but still they are in many extreme environments around the globe. It seems that a clear understanding of their life processes, conditions of occurrence and metabolism may have enormous implications for many fields of human life, including biotechnology, science, and various industries, because it is a group of microorganisms with a high potential.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

DECISION SUPPORT SYSTEMS TO ESTABLISH PLANTATIONS OF ENERGY CROPS ON THE EXAMPLE OF WILLOW (*SALIX VIMINALIS* L.)

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KEYWORDS

technology card, energy willow, wizard, advisory system

ABSTRACT

Presently there is lack of studies or computer programs open for general enabling agriculturists to estimate the cost of establishing the plantation of the energy willow. Present paper meets this demand halfway by creating the framework of software supporting decisions made by agriculturists in the thematic range. The main assumption of the program creating is its accessibility and user-friendliness. Therefore the internet environment has been chosen for it provides unified and straightforward navigation mechanisms and it do not require from the user an extra software installation except any web browser open to public in all operating systems. The objective of this paper is devising the computer program working in the web browser enabling generating the technological card of establishing the energy willow's plantation diversed with regard to the standard of the agricultural field culture, farming area, pre-cultivation endeavours and the method of planting.

INTRODUCTION

An increasing number of companies producing heat or electricity are interested in receiving biomass. This is due to necessity for Poland to adapt to adopted legislation, which determine the amount of energy generated from renewable sources in the country's energy balance of 20% by 2020.

Among renewable energy sources biomass derived from the so-called. Energy crops occupy the main positions. The energetic willow is at the forefront of plants grown for energy purposes. In Poland, the most popular variety is willow *Salix viminalis var. gigantea* or her clones [Faber et all 2009, Dubas et all 2005].

The popularity of this plant is due to many reasons:

- large annual growth – even 14 times faster than natural forest growth,
- high energy value,
- long life span of the plantation, over 25 years,

- *Direct payments are possible*,
- Can be cultivated under a variety of physiographic conditions: in the lowlands and on the slopes, on good and bad soils - fertile and poor, clean and polluted, devastated and degraded both biologically and chemically (military polygons, heaps of aggressive waste) on soils with high and low levels of groundwater. [Kotowski, Dubas 2004]

Polish farmers who want to grow energetic willows face a number of problems, from plant establishment through subsequent plant protection to harvesting and reclamation of plantations.

The decision of the grower to establish a willow plantation depends on many factors such as:

- conditions for establishing a plantation,
- knowledge of production technology,
- available machine park,
- sense (cost-effectiveness) of the project.

Willow plantations for energy purposes can be compared to the long-standing monoculture of traditional agricultural crops. Growing plants in monoculture, regardless of their type, creates the danger of depletion of soil resources. The progressive accumulation of pests leads ultimately to increased damage caused by diseases and pests. [Czysta Energetyka 2005].

At present, there are no publicly available studies or computer programs that allow the farmer to estimate the costs of setting up a willow plantation. This work meets this need by creating software frameworks that support farmer decision making in the thematic area. The main purpose of the program being created is the availability and simplicity of service. Hence the choice fell on the internet environment as it provides unified and simple navigation mechanisms and does not require the user to install any additional software besides any web browser available in any operating system. The aim of the work was to design and create a transparent and accessible computer system (Internet application) working in the Internet environment, supporting the potential grower by the possibility of generating technology cards for the establishment of energy willow plantations.

Establishment of energetic willow plantation

A properly planted plant can be used for a period of 15-20 years and give 14 to 17 tonnes of dry wood per hectare per year [Szczukowski et all 2004].

The soil for willow plantings is prepared taking into account the type of soil and the level of agricultural field culture. Prior to setting up a plantation, it is mandatory to do two treatments – weeding and adjusting the pH of the soil to the needs of the willow. [Dubas et all 2005]

In the year preceding the planting of willows, the most frequently used are agrotechnical treatments such as:

- clearing of trees, bushes,
- cultivating and / or discarding,
- spraying total herbicide to destroy weeds,
- liming or mulching peat soil,
- deep-seated or winter plowing with deeper [Dubas et all 2004, Dubas et all 2005].

One of the first agronomic treatments before planting willow is the grubbing of trees and bushes to clear the surface of the future crop of plants protruding from the surface of the earth and from their roots. Future planters have an access to mechanical and chemical methodologies. Mechanical grubbing methods involve pulling whole trees or bushes, together with roots and aerial parts. Selection of chemical grubbing methods is limited to young trees and shrubs (3-4 years) for the application of glyphosate-containing total herbicides. This measure should be applied on the green list because it works by chlorophyll. In the case of older trees and shrubs, first cut them with a saw and then place a specially prepared mycelium on the stems, which will spread the carp in a few months.

Treated trees, shrubs and weeds should be raked. They must not be burnt on the swath, as this treatment leads to the extinction of biological life in the topsoil of the soil. Sliced plants, if possible, can be used for composting or burning in other places, subject to appropriate fire protection regulations..

Once the future plantation has been cleared, the top layer of the soil should be destroyed, which will create favorable conditions for germination of the weed seeds and cereals, which should later be removed. Plattering is usually done twice, but if this is not enough then cultivating is still required. This is done when undergrowth of willows is prepared for long standing fallow and wasteland.

Approximately two or three weeks after plattering, spraying with the herbicide of the future plantation should be done. As an indicator of the start of spraying is taken to appear in the field of commonly occurring weed *Agropyron repens*. Optimal spraying time is when *Agropyron repens* will release the third or fourth leaf. The farmer has to decide by himself

on the means he will apply for spraying. The recommended dosage and concentration is on the package, but usually it is about 3 to 5 liters per 1 hectare, with 1 liter of solution dissolved in 50 liters of water [Dubas et all 2005].

As previously mentioned, an important step in setting up a willow plantation is to adjust the soil to willow requirements. The optimum reaction of the soil on willow plantations is pH 5.5-6.5 [Gradziuk et all 2003, Różanowski 1993]. For some willow varieties this range may be slightly larger (within 5.5 and 7.5), however, pH above 7.5 is poorly tolerated by the willow [Dubas et all 2004]. The site selected for the willow plantation should be inspected by the staff of the Chemical and Agricultural Station, stating the soil response. If the soil is too acidic, raise its pH by liming with meadow or magnesia. In the opposite situation, when we have to reduce the soil reaction, the soil is acidified with ammonium sulphate or magnesium sulphate [Dubas et all 2005]

It is no less important to adjust the level of nutrients in the soil to the requirements of the willow. Willow demand for fertilizers in the first year of vegetation depends on its variety and is at a level:

- 20 to 30 kg of nitrogen (N),
- 10 to 20 kg of phosphorus (P),
- 20 to 40 kg of potassium (K),

In calculated as clean ingredients per hectare [Dubas et all 2005].

It is more prudent to use spring fertilization because it is possible to use fertilizers whose decomposition and thus the availability of willow is the best. At these activities it is recommended to use professional advice of employees of the Chemical and Agricultural Station or consult the point of trade in fertilizers.

The last exercise done in the autumn should be deep-drawing done to a depth of about 40 cm or deep plowing at a depth of about 30 cm. Both deep-rooting and plowing aim to loosen and aerate the soil. If the soil on which the plantation is planted has been repeatedly plowed at the same depth, the deepening will additionally destroy the resulting plowed sole, but it can only be made where the soil is not stoned.

Treatments most often performed in the spring, before planting willow, it:

- harrowing,
- spraying with herbicides,
- fertilization of new plantings.

In order to level the surface and to retain moisture in the soil, the harrowing or harrowing of the light harrow is performed. Spraying is aimed at destroying perennial weeds that are in the process of germination. There are many chemicals used in this treatment, most often it is Azotop 50 WP in the amount of 1.5 to 2.5kg / ha or Bladex 50WP in the amount of 2 to 5kg / ha , Bladex 500S.C. in the amount of 3 to 4 kg / ha [Tworkowski J. 2006]. The stated amounts of chemicals usually dissolve in 200-300 liters of water. Follow the directions for use of the herbicides labeled on the label. In addition, it is not allowed to take part in plantings before the biological decomposition time expres. This time is indicated on the label. Joining the plant before the expiration of the grace period may damage the germinating cuttings.

The last treatment before planting willow planting is to adapt to the needs of the willow nutrients of the soil. The best solution is to use one of the possible multi-component fertilizers available on the market. It is true that multi-constituent fertilizers are more expensive than one-component fertilizers, but by using them they can be saved on the cost of performing the treatment. In the first year of willow vegetation it is not recommended to use as sludge sludge because it contains too much assimilable nitrogen.

Analysis of system requirements and computational model - methodology

The work was carried out in accordance with the recommendations of software engineering [Sommerville 2003]. The application process was started by analyzing the structure of the advisory system for the establishment of energy plantations and determining the needs and needs of the people to whom this system is mapped to the computerized advisory system.

The analysis allowed to set detailed functional and non-functional requirements of the proposed system:

- the system should include elements that support the decision process (eg, photos, charts, animations),
- the results of the system should be displayed in a clear, transparent manner,
- The system should have the functionality to run it in any web browser without having to install additional components,
- The system should run on mobile devices equipped with a web browser.

Designing thematic applications required the creation of a computational model of the costs and the labor and energy needed to set up the plantation. The project uses a computational model complying with the calculation methodology developed at the Institute

of Agricultural Engineering and Computer Science, University of Agriculture in Cracow. This methodology consists of three stages:

- work calculation,
- calculation of energy expenditure,
- calculation of machine operating costs,
- calculation of material costs.

An example calculation model for willow is shown in the figure 1.

Design and implementation of an advisory system

The next stage of system implementation - after defining the requirements and developing the theoretical basis - is to define its structure in detail, and to analyze and select IT technologies fulfilling all the assumptions. [Płodzień et all 2005].

The structure of the system, its division and linkage between the various elements are shown using the UML class diagram (Fig. 2). The designed models were the basis for the implementation of the application code.

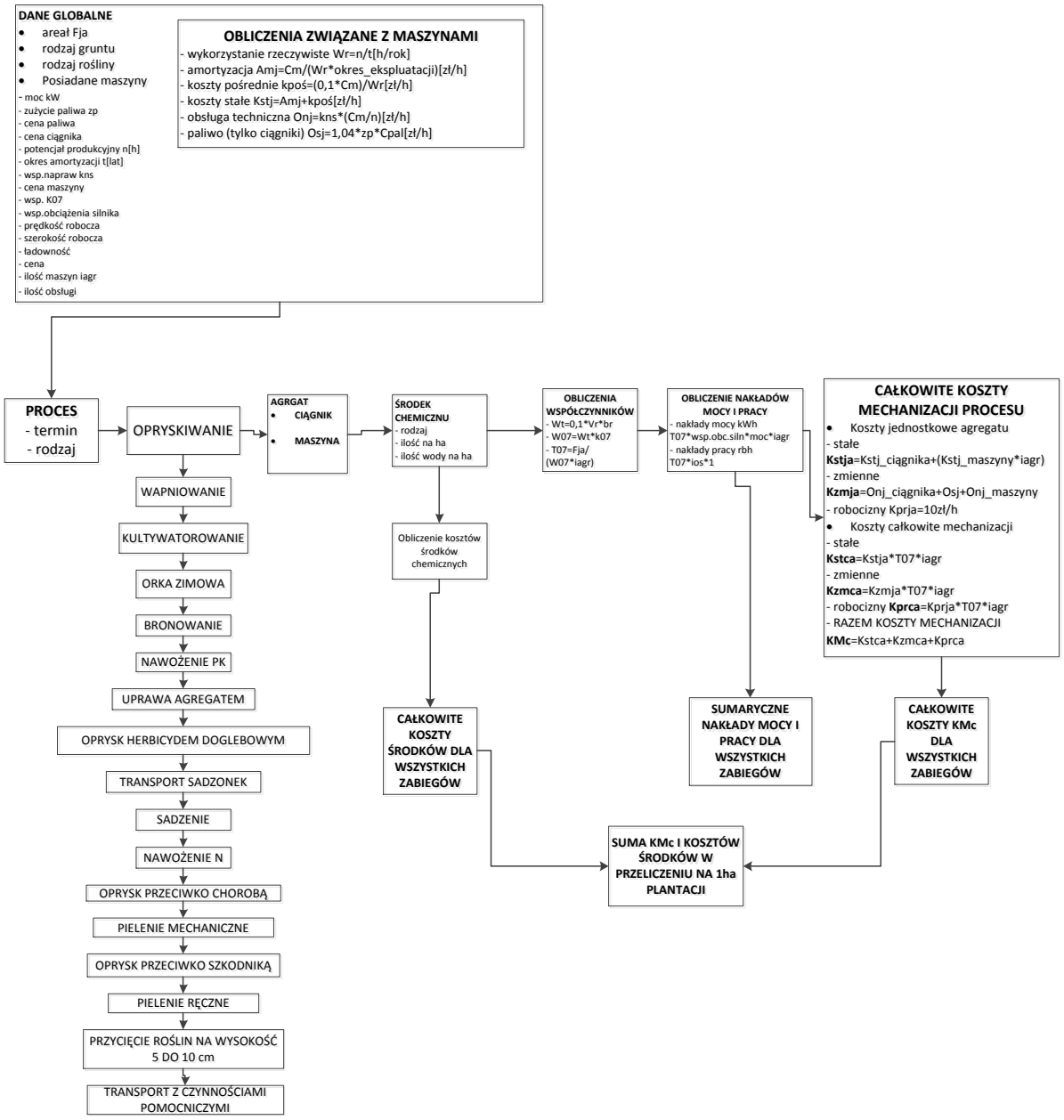

Fig. 1. An example of a computational model for energy willow

Encoding in the chosen environment and programming language is the most important step in developing an application. Based on the assumptions of the design and analysis of the availability and functionality of selected IT solutions, IT technologies were selected meeting these criteria.

The foundation of the whole application is universal languages such as PHP, HTML, JavaScript, CSS and SQL. Thanks to such widespread solutions, the application can be launched on all popular web browsers without having to install additional components.

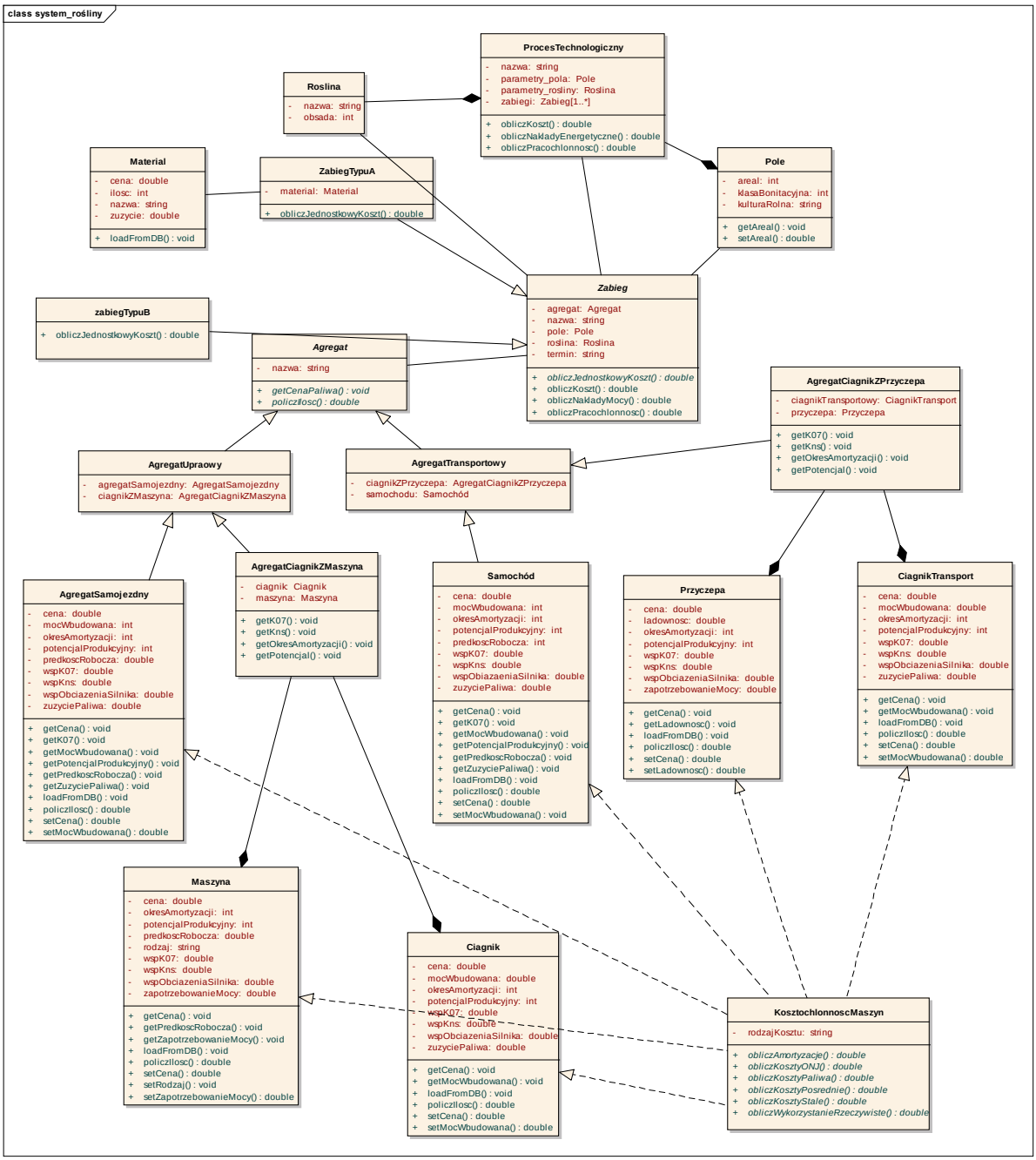

Fig.2. Static structure diagram of the system. Class diagram

The designed advisory system is a client-server technology. The program code, written in PHP, generates another HTML page. Breaking an application into multiple files results in the need for a variable value transfer mechanism between pages. The standard session mechanism implemented in PHP was used. The program code communicates with the database located on the server using SQL queries. The presentation module, which is the main element of the user interface, provides data stored in the database and the current state of progress informing the user of the current step.

The most important element of the designed system is the module generating technology cards for plantation of energetic plants. This card is generated based on the user input and information stored in the database. It is supplemented by information on the total cost and energy expenditure associated with establishing a plantation (fig. 3).

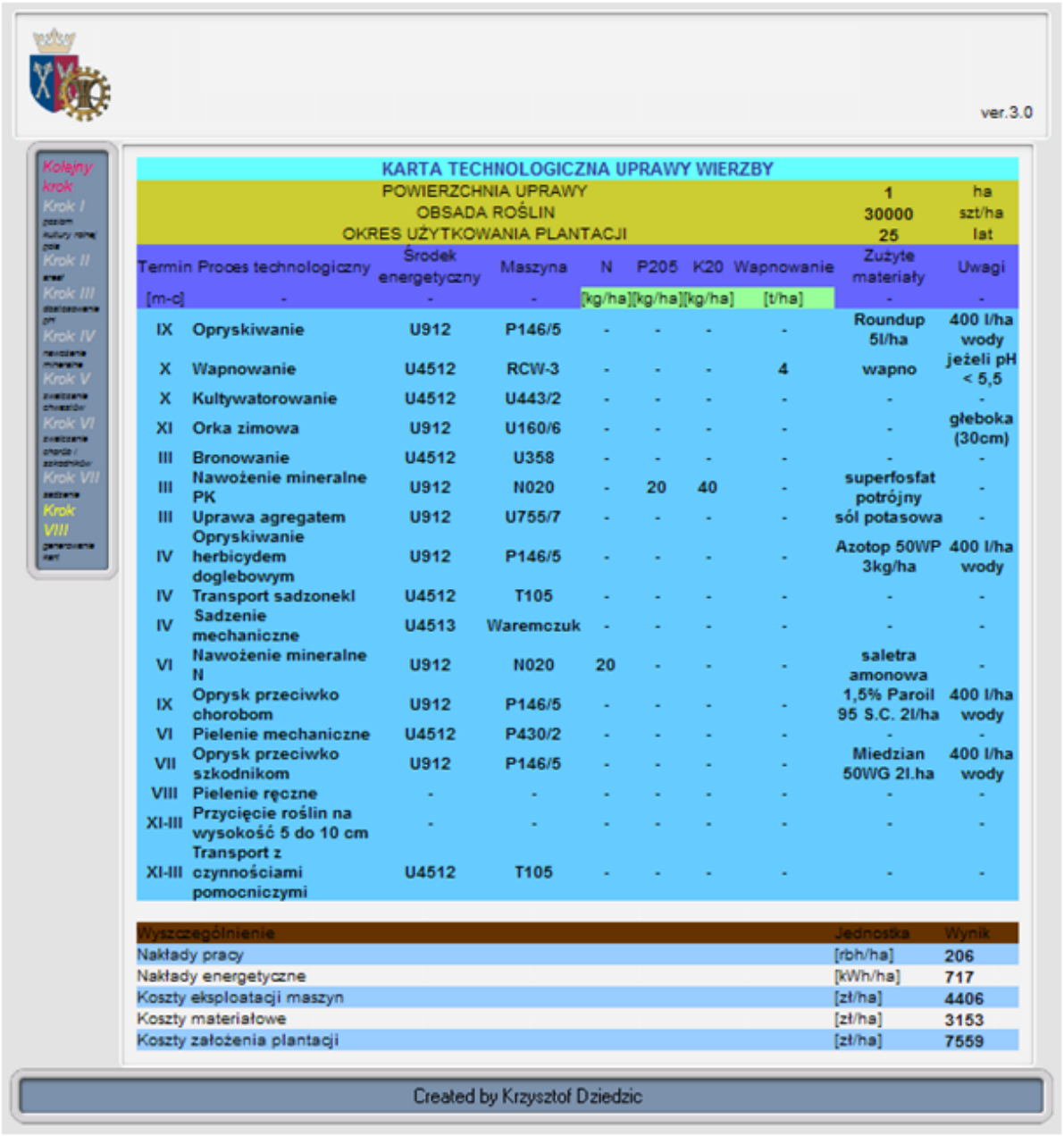

Fig. 3. Technological card for planting willow energy plantations

SUMMARY

The result of the work is the development of an advisory system supporting the potential grower in the decision-making process of establishing energy plantations. The system and technology card wizard provides information on labor costs, energy inputs, machinery costs, material costs and total plantation costs per hectare.

The application includes technological cards that vary in terms of culture level, crop area, planting practices and planting methods.

Applied technologies allow you to run the system on any device equipped with a web browser and Internet access without having to install additional components. Simplicity and intuitiveness of use make it possible to use the system for people who have basic Internet skills.

Application performance is limited to registering user selections organized in forms displayed on 7 screens. The last screen is designed to show the technology card generated from previous selections and view the summary. The user is guided in a clear and unequivocal way between the steps. Such a simplified design of the program interface allows it to be used even if it is not prepared informally for a farmer.

The application does not allow for a precise calculation of plant establishment costs, but only generates sample technology cards that conform to real conditions and their estimation for predefined sets of machines and procedures. An important criterion for its creation was the simplicity of operation - limiting user choices to the required minimum. Hence, the farmer gives only qualitative information on subsequent forms, eg choosing the crop size from arbitrarily established compartments or deciding on mineral fertilization.

It is possible to extend the application in such a way that the user introduces numerical values, selects the machines for the treatments or determines their types independently. In this case, it would be necessary to create the appropriate databases (machines, devices, treatments) and implement detailed algorithms to calculate the costs and inputs (work, energy) of plantation. . It would also require a significant expansion of the graphical user interface allowing for the acquisition of so much information. The application would then not be so intuitive and would target to advanced users who know the specifics of modeled and calculated processes.

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SCIENTIFIC ACHIEVEMENTS IN AGRICULTURAL ENGINEERING AGRONOMY AND VETERINARY MEDICINE

QUALIFICATIONOF GEODETIC AND CARTOGRAPHIC WORKS IN THE AREAS OF MANUFACTURING ENTERPRISES IN THE LEAN MANUFACTURINGASPECT

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Optimization and organization of work, cartographic materials, management, quality system

ABSTRACT

Management of manufacturing companies is a very responsible work. The use of management, planning, logistics and communication tools is possible through high quality products with rational raw material management. However, extensive responsibilities, coupled with the tremendous amount of information and the wide range of work done on the production site, must be supported by external factors to improve the functioning of the company as well as improve productivity and safety. Geodetic works, including inventory of sites and facilities, and cartographic materials for logistical and planning processes may be helpful. An additional advantage may be the introduction of the Lean Manufacturing philosophy in the production management process.

The aim of the study was to determine the usefulness of geodetic and cartographic work in manufacturing companies in the aspect of Lean Manufacturing. Within the framework of work, decision problems have been identified in companies that can be solved in geodetic work. The basic assumptions of slim management have been characterized. Also a quantitative bibliometric analysis of the analyzed issue was carried out. All these treatments have validated the merit of combining the tools offered by geodesy and Lean Manufacturing's philosophy in manufacturing engineering to improve efficiency.

INTRODUCTION

The purpose of this work was to use tools from the geodetic area to optimize production and more specifically to fit into the Lean Manufacturing philosophy. Thanks to the combination of these two areas, it is possible to better control the production and infrastructural parameters and, consequently, to reduce material and labor costs in the long run.

DEFINITIONS

Cartographic materials include maps and technical documentation in their scope, including projections and cross-sections of individual buildings. With their use, it is possible to identify individual buildings belonging to the company. Easy and fast access to graphical information allows to pinpoint any location in production enterprise and thus streamline workflow by organizing a traffic. It also provides comprehensive spatial information about plant and machinery located on site.

Geodetic work is a set of measurement and calculation activities aimed at determining the position of individual points and elements as well as control of geometric constancy of objects. Measurements are made with electronic timers, tachymeters, the odolites and

modern tools such as laser scanners. In manufacturing facilities, the vast array of equipment, tools, machinery and equipment means that the main components of their construction must be adequately controlled for safety, efficiency and productivity. Observation of component geometries allows them to reduce their operation - which increases their reliability and availability, thus reducing the cost of any repairs and minimizing production downtime.

Measurement of deformation determining and deformation of objects is one of the basic tasks in engineering geodesy. The study of constancy of object geometry and the degree of variation in their structure includes a number of observations and calculations to determine possible changes. Construction and equipment deformations are analyzed by measuring and evaluating the displacement of checkpoints (Bernasik i Mikrut 2007). One of the most frequently observed equipment in manufacturing plants is crane structures. The crane track observation and its regulation are based on the control measurements to determine the actual geometric state of the track and the other load bearing structures. The assessment of the suitability of the roadway for further operation consists in comparing the condition of the elements with the normative state. Further rectification adjustments are made to adjust the shape of the structural members in accordance with the relevant technical conditions. (Przewłocki 2013). All these treatments ensure the safety of work and improve its quality, which translates into productivity.

USING GEODESIC WORKS AND MEASUREMENTS IN A MANUFACTURING COMPANY

A lot of geodetic work is being carried out for inventory purposes of components of manufacturing plants. The analyzes and regulations conducted on them support the work of individual machines and equipment, thus increasing their endurance for the operation, the efficiency of the operation of the equipment and the safety of the users.

The purpose of all measurements is to investigate the reality of objects by controlling the correctness of geometric dimensions and their location in space. Geodetic measurements also provide information about the entire facility or plant. This information is used for planning and organizational purposes. During the process of design and execution of modernization and construction works it control the formation of geometrically correct elements of buildings. Such observations are also used in the processes of depositing and assembling components of machines and devices.

Another element of measurement is the control of the creation of objects produced in this one plant. Large and complex items often have to have specific and highly

standardized geometric dimensions. Production control is carried out by appropriate geodetic or photogrammetric measurements. The purpose of this measurement is to investigate the facts that underlie the technical diagnosis and to determine the admissibility of the facility. (Zawieska 2010).

CARTOGRAPHIC MATERIALS AND TECHNICAL DOCUMENTATION OF PRODUCTION ENTERPRISES

Production companies include buildings and structures including technical equipment and transport and access roads to buildings. All these elements are standardized and normalized on maps. Industrial buildings are represented on the base maps by tracing their outline and assigning the appropriate "p" sign. This building is defined as: ”*The building is designed for production purposes for all industries, in particular: factories, workshops, production halls, slaughterhouses, breweries, assemblers, film studios etc.*.” (Regulation of the Minister of the Interior and Administration, 2011). Very often in this type of specialized cartographic studies work aimed at generalizing objects on the map to expose important content relevant to the production. Many maps include only terrestrial network and buildings located on the site.

Managing of extensive production areas requires reliable and unequivocal information about each of its locations. Individual buildings have drawings of projections and sections that provide information about the production area. Sketch of the position of the components of the enterprise gives an additional insight into the location of machines and equipment incorporated into the enterprise. This information is supplemented with a description of the technical parameters and a technical drawing of the construction of the equipment gives a comprehensive information about the objects and equipment.

Each manufacturing company should have a documentation of the location of all buildings and projections and crossings of individual floors and rooms. This information is used in a variety of ways, but is also available to people working in industry and industry professionals, it have the following functions:

- information: indicating the location of individual rooms / buildings
- space management: the basis for the creation or modernization of existing production sites
- planning and design: design, change and investment: construction, extension, reconstruction or demolition of relevant parts of buildings on the premises

- improve transport and accessibility: By designating the most efficient communication routes in a manner that does not interfere with production
- for protection: indicates escape routes in situations endangering health and life

LEAN MANUFACTURING

Production companies are a specific object in the context of management.

According to the definition proposed by the Encyclopedia of Lean Manufacturing Management: "(...) *It is a production management system in a manufacturing company that is designed to reduce waste and eliminate unnecessary operations and procedures in the production process, while providing products and services of the highest quality expected by customers while maintaining low production costs and using relatively low raw materials."* [https://mfiles.pl/pl/index.php/Lean_manufacturing].

This system has revolutionized the approach to the production management. The introduction of this system can help reduce losses, significantly reduce production time and, consequently, increase productivity while increasing profits. Priority in this field is Toyota Motor Company, which has had the greatest impact on the implementation of this philosophy in manufacturing practice. Since the success of the Japanese company, the concept of "continuous improvement" has rapidly spread to the rest of the world. This system is based on the definition of product value, the organization of the stream value. It is guaranteeing the flow of value through the stream value, forcing a continuous reaction of the system to the needs of the customer and ending with the basic element, i.e. continuous improvement. This improvement applies to every element of the production chain. Care should be taken to: the condition of the machines, the provision of high quality finished products, the training of staff and senior management. Properly implemented and maintained system drives the company and gradually reduces the irregularities resulting from the production process. Such a system should grow with the company. The idea is to gradually implement more tools from Lean Manufacturing.

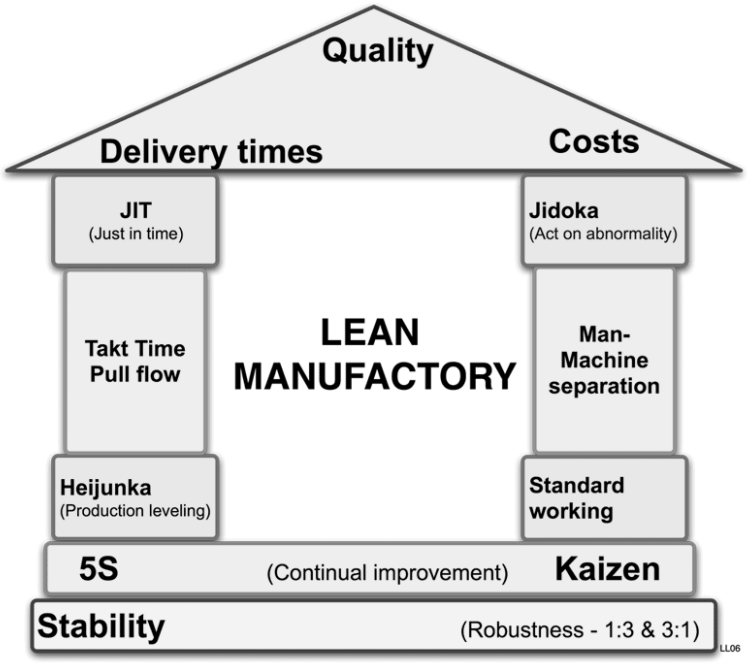

Fig. 1. Lean manufacturing system model.

Source: Womack et al. 2003

Within Lean Manufacturing aspect is possible to distinguish many methods and tools that support the entire system (Fig. 1). Thanks to solid foundations, this philosophy can bring significant profits to companies.

In order to function properly, aspects of Lean Manufacturing should be implemented on a broader scale than the enterprise itself. The biggest effects can be achieved by implementing it throughout the supply chain, from the supply company, through the warehouses to the distributors (Pomietlorz 2015).

LITERATURE ANALYSIS OF OPTIMIZED AREAS

A simplified bibliometric analysis was also included in the study. A search was made in the Web of Science database - Core Collection in English-language articles from 1945 to 2016 for indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC. Search terms were: 'geodetic work' and 'lean manufacturing'. The search areas were narrowed to the following categories Web of Science: ENGINEERING MANUFACTURING and GEOSCIENCES MULTIDISCIPLINARY.

Bibliometric quantitative analysis included the number of published papers and the number of citations in each year.

In particular, the analysis of a set of documents allows to distinguish several periods (chart 1):

- first: 1994-2004 - the average annual number of publications is = 4,46,
- second: 2005-2010 - the average annual number of publications is = 15,33,
- third2011-2016 - the average annual number of publications is = 31,00.

By analyzing chart 1, there is a noticeable increase in the average annual number of publications in each successive period.

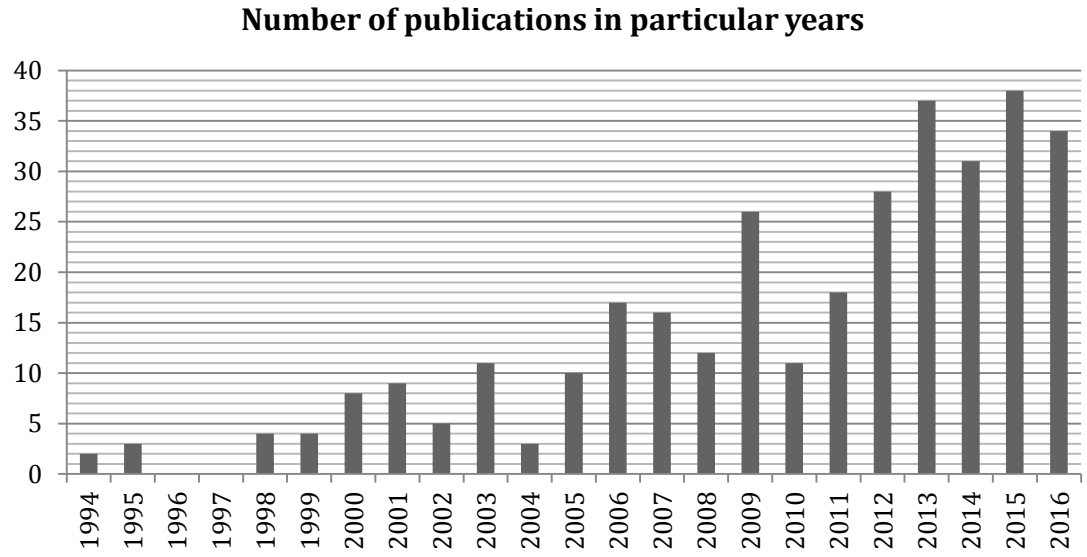

Chart 1.Number of publications in particular years.

source: own study

The next parameter to be analyzed is the number of citations. From Chart 2 (Figure 2), a significant increase in the number of citations occurred in 2007.The highest number of citations was recorded in the period 2013-2016.Also among the most cited documents, lean manufacturing is dominant.

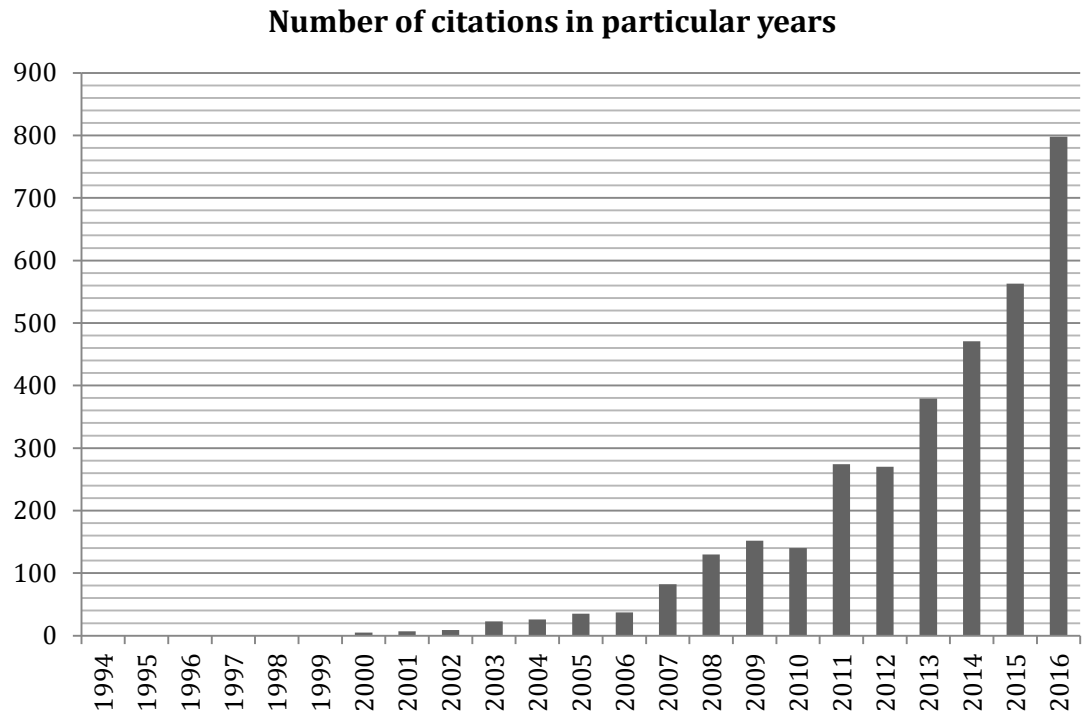

Chart 2.Number of citations in particular years.

source: own study

Tab. 1. Summary of the highest number of citations in the search area in the years 1994-2016.

*(TC- Total number of citations)

AUTHORS	TITLE	MAGAZINE	TC
Naylor, JB; Naim, MM; Berry, D	Leagility: Integrating the lean and agile manufacturing paradigms in the total supply chain	INTERNATIONAL JOURNAL OF PRODUCTION ECONOMICS	382
Abdulmalek, Fawaz A.; Rajgopal, Jayant	Analyzing the benefits of lean manufacturing and value stream mapping via simulation: A process sector case study	INTERNATIONAL JOURNAL OF PRODUCTION ECONOMICS	174
Yang, Ma Ga (Mark); Hong, Paul; Modi, Sachin B.	Impact of lean manufacturing and environmental management on business performance: An empirical study of manufacturing firms	INTERNATIONAL JOURNAL OF PRODUCTION ECONOMICS	140
Mason-Jones, R; Naylor, B; Towill, DR	Lean, agile or leagile Matching your supply chain to the marketplace	INTERNATIONAL JOURNAL OF PRODUCTION ECONOMICS	126

source: own study

There were 338 documents in the separate collection. They consisted of 216 scientific articles, 121 post-conference materials, 8 reviews and 7 notes.

Most of the documents were created in the following countries: USA - 105, Indi - 40, Great Britain - 34, Brazil 17, Italy and Malaysia - 16 and Turkey 13. Other countries have issued 10 and fewer documents in the search area in those years. Among the research centers in the top five search results were: National Institute Technology Tiruchirappalli - 9, Universidad Federal de Sao Carlos - 7, University Of Michigan - 6, University Of Michigan System - 6, University Of North Carolina - 6.

Most of the documents in the search area were written by: Vinodh S. - 11, Godinho M. - 7, Kodali R. and Tiwari M. - 4.

The number of documents by Web of Science category relative to the category and the number of documents falling into it is as follows:

- Engineering Manufacturing - 331
- Engineering Industrial – 174
- Operations Research Management Science– 142
- Automation Control Systems - 53
- Engineering Mechanical - 36
- Computer Science Interdisciplinary Applications - 26
- Materials Science Multidisciplinary - 19
- Computer Science Artificial Intelligence - 10
- Robotics - 10
- Engineering Electrical Electronic - 8

By analyzing the number of documents classified by the Web of Science category, it is possible to notice that most documents fall into categories: ENGINEERING MANUFACTURING, ENGINEERING INDUSTRIAL, OPERATIONS RESEARCH MANAGEMENT SCIENCE, AUTOMATION CONTROL SYSTEMS. All major areas are within Lean Manufacturing. It is visible that none of the geodesic subdivisions was in the main ranking.

CONCLUSIONS

Using Lean Manufacturing tools in common with relevant geodetic and cartographic documentation gives you the ability to efficiently manage your production company. The paper presents common areas where research into the implementation of the proposed analyzes and solutions can be carried out in the future.

The conducted quantitative bibliometric analysis showed that the presented problem is current. The trend of increasing the number of publications and citations indicates a growing interest of the scientific community in this topic. The number of solutions and implementations of the elements presented in the articles in relation to the management forms can produce effective profits for the manufacturing companies.

Geodetic work at the production site ensures the control of the actual state of the machinery and equipment by assessing the constancy of the geometry of the components. Testing the displacement and deformation of the structure from the connection with appropriate maintenance and repair works ensures their correct and long-term operation.

Cartographic materials of manufacturing companies provide spatial information about the facility and its equipment. They perform a variety of functions, thus providing the opportunity for development, increasing efficiency and productivity and safeguarding the safety of people on site. Documentation is also the basis for investment planning and the forthcoming modernization of the plant in the area.

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