Effect of milk from the cows, receiving herbal extracts in their diet on homeostasis of laboratory animals being a model for human

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The aim of the study was to examine whether extracts from herbal preparation Herbatan and of *Echinacea purpurea,* being employed as feed additives for dairy cows, and being added directly to milk, affected its functional value. The studies were conducted on Wistar rats, at random classified in 6 groups, 10 animals in each group. The rats from group 1 received milk from the cows which received addition of 150 ml of Herbatan in their diet. The milk for the rats from group 2 derived from the cows fed the diet with the addition of *Echinacea purpurea* extract (8.6 mg/kg of body weight). In group 3, the animals received 400 µl of Herbatan in their daily ration of milk; in group 4, the rats received the addition of 1.13 ml of *Echinacea purpurea* extract. The rats in all group received 55 ml of milk per head, in average. The animals of group 5 received milk without herbal extracts and those ones from group 6 were fed the synthetic diet. The following determinations were carried out: body weight, relative body weight of the rats, the selected biochemical parameters in blood serum: TP, ALB, BUN, GLU, TRI, CHOL, HDL, LDL, VLDL, AST and ALT, morphological parameters of red and white cell system, the level of the selected immunological indicators: immunoglobulins IgG, IgE, IgM, Il-2 and Il-4 in blood serum of the rats, phagocytic activity of monocytes and neutrophils of peripheral blood. Histological measurements of intestinal villi were carried out. The effect of herbal extracts on functional value of milk was reflected in: lower content of cholesterol and LDL in the rats fed the milk diet with the direct addition of Herbatan and *Echinacea purpurea* to the milk as compared to the rats from the remaining groups, in the highest level of erythrocytes and hemoglobin in the rats receiving the milk from the cows fed with the addition of Herbatan, in the intensive fluorescent effect of phagocytic neutrophils in the group of the rats receiving the milk from the cows fed the diet with Herbatan addition and in the group of the rats which received the addition of *Echinacea purpurea* directly to milk.

KEY WORDS: herbal extracts / milk / functional value / rats

Feed additives, being presently employed in nutrition of farm animals, include also herbs. Due to a wide spectrum of bioactive compounds, herbs may affect positively or negatively the condition and health of animals and also, the sensory and functional properties of food products of animal origin. It is dependent not only on the appropriate selection of herbs, used as feed additives but also on level of their rate in the diet.

Herbatan is an herbal extract employed in practice as the additive to diet of dairy cows. The herbs which are found in the composition of Herbatan include extracts from the following herbs: *Aloe vera* (true aloe), *Lepidium meyenii* (maca), *Taraxacum officinale* (common dandelion), *Eugenia caryophyllata* (clove tree), *Humulus lupulus* (common hop), *Cinnamomum* (cinnamon tree), cinnamanaldehyde, *Castanea sativa* Mill (sweet chestnut tree), organic acids: malic, lactic and acetic acid. Herbatan contains the following biologically active substances: polysaccharides and lectins in aloe, alkamides in maca, flavonoids and tannins in hop, clove tree, sweet chestnut tree and in common dandelion. Clove tree, hop and cinnamon tree contain also essential oils and besides it, clove tree possesses also a very strong antioxidant – eugenol.

Echinacea purpurea is employed in human medicine in order to increase the immunity of organism; it may be also used as feed additive in a dry or extract form in nutrition of dairy cows [12], in nutrition of goats and calves [35] and of calves [30]. Bioactive substances, being found in *Echinacea purpurea* include mainly: polysaccharides [42], alkamides [8, 34] and also, derivates of caffeic acid, i.e. cichoric and kaftaric acid [14, 33]. All the mentioned above components of Herbatan and *Echinacea purpurea* exert the influence on the functions of immunological system. Polysaccharides participate actively in immunomodulation [43]. They may stimulate specific and nonspecific immunity; modulate functioning and acting of the particular elements of immunological system in general and on the cellular level. Lectins induce immunological system in the early stage of infection [6]. Flavonoids act as antioxidant, they bind free radicals. They reveal also immunity-supporting activity via, *inter alia*, the effect on increase of T lymphocytes' production [29]; they affect favourably the phagocytic capability of leukocytes and on the rise of the level of monocytes and granulocytes of peripheral blood [22]. Besides it, they have the anti-inflammatory effect and inhibit biosynthesis of prostaglandins, superoxide anions and leukotrienes [23]. Essential oils have antibacterial, viricidal, fungicidal and anti-inflammatory effect [20]. Eugenol is a strongly acting antioxidant, having also bactericidal and antiviral effect [37]. Each of the bioactive components of *Echinacea purpurea* (polysaccharides, alkamides, derivates of caffeic acid: cichoric and kaftaric acid) reveal immunostimulating activity [14, 33]. The best effect was found in case of the simultaneous application of polysaccharides, alkamides, and cichoric and kaftaric acids [18, 21].

In the studies on laboratory animals – rats and mice – the effect of *Echinacea purpurea* on the increase of cellular activity connected with the increase of the number of lymphocytes [15, 39, 41] was observed; also, the rise in the phagocytic activity of mononuclear cells of peripheral blood, mainly of monocytes, neutrophils and granulocytes [17, 18, 31, 39], on the growth of cytokines' production; -1alpha, IL-1beta, IL-2, IL-6, IL-10 TNF, INF-gamma [3, 4, 16, 36] and the increased production of antibodies, was recorded.

More and more frequently, the contemporary consumer pays attention to functional properties of animal-origin products such as milk, meat and eggs. The effect of the products of animal origin, being considered as functional ones, on homeostasis of organism due to the application of appropriate feed additives (here: herbal extracts) in animal nutrition may be evaluated in the tests on laboratory animals (being considered as model for human in respect of the experiments in the field of nutritional and medicinal sciences) via determination of blood morphological parameters, blood biochemical indices as well as indicators of the immunity level.

The aim of the study was to examine whether the extracts of herbal preparation Herbatan and *Echinacea purpurea*, being employed as feed additives for dairy cows and being administered directly to milk, affected its functional value.

The research hypothesis assumes that herbal additives: Herbatan and *Echinacea purpurea* which reveal anti-inflammatory effect by decrease of the somatic cell count in the milk of cows and goats fed the diets with the participation of the mentioned herbs [26, 35] and, also in case of *Echinacea purpurea*, affect the immunity via the increase of the level of immunoglobulins: IgG [12] and IgM [30] in blood serum of cows and calves, may also affect favorably functional value of milk.

Material and methods

The studies were carried out on Wistar rats with the average body weight of 88.86 g. After the adaptation period, lasting for 5 days, during which all the rats received a synthetic diet and water *ad libitum*, they were at random divided into 6 groups, 10 animals in each group. The specific experiments were conducted for 21 days in case of the diets with the participation of *Echinacea purpurea* and 90 days in case of the diet with the application of Herbatan. During the experiment, the rats were fed according to the scheme, presented in table 1.

Table 1
Experimental design

Diet of rats					
Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic
Herbatan 150 ml	*Echinacea purpurea* 8.6 mg/kg of body weight	Herbatan (400 ml)	*Echinacea purpurea* (1.13 ml)		
group 1	group 2	group 3	group 4	group 5	group 6

Milk derived from Stogi farm, from Polish Holstein-Friesian cows; the mean milk yield was equal to 7900 kg of milk per lactation. The content of fat and protein and somatic cell count in the milk was 3.8%, 3.4% and 106.6 x10^3, respectively.

The milk for the rats of group 1 came from the cows, which obtained the addition of 150 ml of Herbatan in their diet.

The milk for the rats of group 2 derived from the cows, receiving 8.6 mg of *Echinacea purpurea* extract per one kg of body weight. Ethanol extract of *Echinacea purpurea* herb, appropriate for the cows with body weight of 600 kg was prepared in single containers for each cow in the group and for each day of the experiment; it was conducted at the Institute of Natural Fibres and Herbal Plants in Poznań, where its rate was also established according to the content of active substances – in this case, it was 8.6 mg of the determined active substances per one kg of cow body weight. Extract of *Echinacea purpurea* was dosed in such quantities as in human medicine, as calculated into 1 kg of body weight. Herbatan (400 µl) in group 3 and extract of *Echinacea purpurea* (1.13 ml) in group 4 were added to daily milk ration for each rat. The rats of group 5, receiving milk without herbal extracts and the animals from group 6, receiving synthetic diet, were the control groups.

The mean daily milk intake by a rat amounted to ca. 55 ml, with the unlimited access to water. The application of exclusively milk diet aimed at stating how active substances, as being contained in *Echinacea purpurea* and in Herbatan preparation and administered indirectly via the milk from cows which were fed by addition of herbal extracts, as well as being added directly to the milk for rats, affected the homeostasis of laboratory animals. The employment of synthetic mixture in the diet and milk with herbal extracts could result in the intake of incomplete quantity of the administered milk. In the experiment, the rats consumed the whole milk ration.

In the experiment, the following determinations were performed: body weight and relative body weight of the rats (comparing the weight of a single individual and the average body weight in the group), the weight of the selected internal organs and relative weight of the mentioned organs (as % of body weight). Histological measurements were conducted under Nikon-Eclipse microscope and cooperating Nikon Digital Sight Ds-U1 camera (Olympus), using program NIS elements; the following parameters were determined: height and width of intestinal villi, depth of crypts, height of muscular and mucous layer and the number of mucous cells. The selected biochemical indicators in blood serum: TP, ALB, BUN, GLU, TRI, CHOL, HDL, LDL, VLDL, AST and ALT were determined using biochemical analyzer Vitros DT 60 II and ready slides of ICN company. Morphological parameters of blood were performed by standard laboratory methods, using hematological analyzer ABACUS.

To determine the level of immunological parameters in rats, the immuno-stimulation LPS (Sigma-Aldrich) for 12 hours before euthanasia of animals was performed. After the application of LPS immuno-stimulation (Sigma-Aldrich), the level of the following selected immunological parameters: IgG, IgE, IgM in blood serum was determined by chemiluminescence method, using Immulete 2000 analyzer, with the application of ready kits of R&D company and measurement of absorption at wavelength of 405 nm, by spectrophotometer Infnitive 2000; interleukin IL-2 and IL-4 in blood serum of rats and phagocytic activity of monocytes and neutrophils of peripheral blood was determined using kit of Orphagen company, using hematoxic factor *E. coli*; the measurement was carried out on in-fow cytometer.

The results of the tests were subject to statistical analysis by variance ANOVA method, in single-factor system, using Statgraphies 6.0 Plus 54.

Results and discussion

Table 2 contains the data concerning initial body weight, fnal body weight, total gain of body weight and relative body weight of the rats. Statistically signifcant differences in values of the examined parameters i.e. fnal body weight and body weight gains and also, relative body weight occurred between the rats from group 6 which received synthetic diet and the rats from the fve remaining groups. It results from the higher energetic value of synthetic mixture in comparison to milk diet. Energetic value of synthetic diet in group 6 exceeded the requirements of animals what is confrmed by signifcantly higher glucose concentration in blood serum in the rats of group 6 – the mentioned levels exceeded the reference values according to Wolfhenson and Lloyd [45]. In case of the rats from the remaining groups, glucose concentration was consistent with the reference values. Any effect of herbs on the gains of total or relative body weight was not found what is indicated by the lack of signifcant differences between groups 1, 2, 3, 4 and group 5. In their experiments as well as in their own studies, many authors have not recorded the effect of herbs on body weight gains of the laboratory animals, e.g. Dalsenter et al. [10], Burnett et al. [5], Shamaan et al. [38], Gupta et al. [19] and Chung et al. [7]. Some authors observed decline of body weight resulting from the decreased feed intake due to a bitter taste of herbs [44].

Table 2
Total body weight gain and relative body weight of the rats

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
Initial body weight (g)	94.20	91.75	87.90	89.78	87.27	82.23	10.60	0.051
Final body weight (g)	149.96[a]	151.57[a]	151.03[a]	148.80[a]	154.04[a]	164.61[b]	14.46	0.020
Body weight gain (g)	55.76[a]	59.85[a]	63.03[a]	59.07[a]	66.77[a]	82.38[b]	10.23	0.020
Relative body weight (g)	145.67[a]	150.24[a]	149.24[a]	146.58[a]	152.78[a]	160.34[b]	10.21	0.051

Mean values of indicators in the rows, marked with pairs of letters differ statistically at $p<0.05$

The increase in body weight of the rats was recorded by Erwanger and Cooper [13] in the experiment with the application of African potato tuber (*Hypoxishem erocallidaea*) being considered as medicinal plant in the diet of the rats.

In table 3, the weight of internal organs: heart, liver, spleen, thymus and kidneys of the rats has been presented. Any statistically signifcant differences in the weight of internal organs between the rats from group 6, fed the synthetic diet, from group 5 receiving milk without herbal additives and the rats from the groups which received milk from the cows fed the diet with the addition of extract of Herbatan and *Echinacea purpurea* (groups 1 and 2) and between the rats from the groups where the addition of extract of Herbatan and *Echinacea purpurea* was administrated directly to the milk (group 3 and 4), have not been found.

Similarly as in their own studies, Dalsenter [10], Li et al. [25] and Makbu et al. [27] did not observe the infuence of bioactive herbal compounds on the weight of internal organs of laboratory animals. On the other hand, Wolf and Weisbrode [44] recorded a lower weight of internal organs of the rats which received extract as compared to the animals from the control group, with the simultaneous decline of body weight of the experimental animals as a result of lower feed intake.

Table 3
Mean weight of internal organs (g)

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
Heart	0.77	0.77	0.78	0.77	0.80	0.77	0.07	0.45
Liver	4.34	4.28	3.95	4.15	4.26	4.36	0.46	0.18
Spleen	0.22	0.22	0.25	0.24	0.20	0.24	0.09	0.23
Thymus	0.16	0.14	0.15	0.16	0.12	0.16	0.08	0.94
Kidneys	0.64	0.56	0.60	0.62	0.56	0.54	0.18	0.38

The selected morphometric parameters are given in table 4.

Any statistically confrmed differences between the examined morphometric parameters of the intestines as affected by the employed herbal additives have not been recorded. The lack of the infuence of active components of the diet on morphometric parameters were also reported by Lai et al. [24]. In the available literature, we may fnd the results of he studies where the authors revealed the effect of active components, being present in

Table 4
The mean values of the examined morphometric parameters of the rats' intestines

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
Height of intestinal villi (µm)	545.69	558.26	532.28	529.66	528.90	543.35	89.54	0.42
Width of villi (µm)	66.33	68.91	66.44	64.32	65.03	71.22	25.89	0.45
Depth of crypts (µm)	132.34	118.81	132.56	110.24	123.45	116.57	32.69	0.38
Height of mucous layer (µm)	144.31	119.98	159.61	118.91	146.78	132.83	79.45	0.46
Height of muscular layer (µm)	72.73	67.13	64.79	66.91	68.83	70.82	32.64	0.54
Number of mucous cells (pcs.)	24	25	26	26	27	29	7.64	0.27

the composition of the diet, on morphometric parameters of the intestines of the rats (e.g. [21]), with the application of polysaccharides in the diet for the rats. The mentioned relationships between the examined morphometric parameters and active substances are also indicated by the studies, *inter alia*, of Domenghini et al. [11]. McAnuff et al. [28] suggest that active substances of herbs may modify the permeability of mucosal membrane of the rats' intestines and by this, decrease or increase the capacity of intake of the nutritive components what causes increase or decrease of intestinal villi. In their studies, Bodiga et al. [2] and Palanch and Alvarez [32] showed that defcient diet affected the decrease of the height of intestinal villi and was the evidence of domination of apoptic processes as compared to the proliferating ones.

Table 5 represents the data concerning the selected biochemical indicators in blood serum of the rats.

Table 5
The selected biochemical parameters of the rats' blood

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
1	2	3	4	5	6	7	8	9
Total protein TP ($g \cdot l^{-1}$)	51.00	48.66	50.57	48.75	50.83	52.00	3.59	0.83
Albumin ALB ($g \cdot l^{-1}$)	31.28	29.00	30.85	31.00	30.33	31.75	2.29	0.68
Urea nitrogen BUN ($mmol \cdot l^{-1}$)	6.05	6.01	7.54	5.94	6.11	7.20	2.25	0.86
Glucose GLU ($mmol \cdot l^{-1}$)	7.07^{a}	7.21^{a}	6.50^{a}	7.22^{ab}	6.58^{a}	8.52^{b}	1.14	0.019
Triglycerides TRI ($mmol \cdot l^{-1}$)	0.74	0.67	0.65	0.60	0.62	0.59	0.13	0.81
Cholesterol CHOL ($mmol \cdot l^{-1}$)	2.18^{ab}	2.74^{b}	1.93^{a}	1.85^{a}	2.48^{ab}	2.74^{b}	0.50	0.019
High density protein cholesterol HDL ($mmol \cdot l^{-1}$)	0.94	1.21	0.92	0.95	1.11	1.07	0.26	0.78

1	2	3	4	5	6	7	8	9
Low density protein cholesterol LDL (mmol·l^{-1})	0.95^{ab}	1.18^{ab}	0.71^{a}	0.50^{a}	1.08^{ab}	1.40^{b}	0.44	0.012
Very low density cholesterol VLDL (mmol·l^{-1})	0.30	0.32	0.30	0.24	0.28	0.26	0.06	0.89
Aspartate aminotransferase AST (U·l^{-1})	208.00	232.00	235.00	279.00	327.00	360.00	160.05	0.56
Alanine aminotransferase ALT (U·l^{-1})	30.50	36.50	32.20	49.50	36.20	37.70	14.47	0.72

Mean value of indicators In the rows marked with pairs of letters a, b differ statistically at $p \leq 0.05$

Statistically signifcant differences between the groups of the rats were found in respect of concentration of glucose, cholesterol and low-density lipoproteins. The highest glucose concentration was recorded in blood serum of the rats in group 6 (exceeding the reference values according to Wolfensohn and Lloyd [45]. In group 6, signifcantly higher body weight gains were also obtained as compared to body weight gains of the rats from the remaining groups (tab. 2) what indicates that synthetic diet exceeded the energetic requirements of the animals. The rats from group 3 and 4, fed the diet with the direct addition of herbal preparations to the milk, were characterized by signifcantly lower cholesterol and low-density protein cholesterol content. Statistically signifcant lower differences were found in respect of the rats which received synthetic diet (group 6) and milk diet (group 5) and the animals, receiving the milk from the cows which were fed with the addition of herbal extracts (group 1 and 2). The rats which received the synthetic diet had the highest level of low-density protein cholesterol (the differences were statistically confrmed) and of the total cholesterol (excluding the rats from group 2).

Signifcantly lower concentration of total cholesterol as well as of LDL in the groups, receiving herbal extracts directly to milk, may result from the effect of active substances, present in extract of *Echinacea purpurea* and Herbatan, on metabolism of cholesterol in liver; they affected more strongly due to direct addition to the milk. The discussed effect may be generated via inhibition of the activity of enzyme - reductase HMGCoA which controls the process of cholesterol synthesis.

We should pay attention to TP and ALB content in blood serum of the rats which were found below reference values according to Wolfensohn and Lloyd [45]. The concentration of TP and ALB seems to be unclear as being found below the reference values for the rats in all groups. If the discussed differences had occurred between the experimental and control groups, we would suspect negative or positive effect of the herbs on metabolism of proteins. Also, ALT values in blood serum of the rats of group 1 and 3 occurred below the reference values whereas AST values were found above the mentioned values in blood serum of the rats from control groups 5 and 6. The authors of the reference standards, Wolfensohn and Lloyd [45] emphasize that the differences obtained in the results may occur and they are not always connected with the disturbances of homeostasis in laboratory animals but may be caused, *inter alia*, by different age and origin of animals or the application of different methods for determination of biochemical parameters.

Table 6
Blood morphology – elements of red blood corpuscle system

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
Erythrocytes RBC ($T \cdot l^{-1}$)	9.144^{A}	8.445^{AB}	7.698^{B}	7.780^{B}	8.060^{AB}	8.590^{AB}	0.73	0.011
Hemoglobin HGB ($G \cdot l^{-1}$)	15.08^{A}	14.18^{AB}	13.22^{B}	13.55^{B}	14.05^{AB}	14.93^{AB}	3.33	0.010
Hematocrit HTC (%)	46.91^{a}	43.48^{ab}	39.66^{b}	41.25^{ab}	42.51^{a}	46.00^{a}	3.33	0.048
Mean corpuscle volume MCV	51.60	50.60	51.80	51.20	54.00	53.00	1.69	0.510

Mean values of indicators in the rows marked with pairs of letters a, b differ statistically at $p \leq 0.05$
Mean values of indicators in the rows marked with pairs of letters A, B differ statistically at $p \leq 0.01$

The data from table 6 represent the elements of blood red corpuscle system in blood of the rats of the experimental and control groups.

All values of the determined elements of blood red corpuscle system did not differ from the values of reference standards adopted for the rats [45]. The rats, receiving the milk from the cows fed with the addition of Herbatan (group 1) were characterized by the highest content of erythrocytes in hemoglobin. Signifcantly lower levels of erythrocytes and hemoglobin in blood serum of the rats were recorded in the groups, receiving direct herbal additive to milk (Herbatan in group 3 and *Echinacea purpurea* in group 4) in relation to group 1 and 2 and in relation to the rats from the control groups 5 and 6.

The data contained in table 7 concern the contents of the elements of blood white corpuscle system in the blood of the rats.

Table 7
Blood morphology – elements of white blood corpuscle system

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
Leukocytes WBC ($G \cdot l^{-1}$)	4.48	4.01	4.15	3.02	4.18	3.98	1.56	0.92
Lymphocytes LIMF (%)	81.00^{A}	82.46^{A}	84.82^{A}	51.00^{B}	89.42^{A}	54.66^{B}	11.81	0.01

Mean values of indicators in the rows, marked with pairs of letters differ statistically at $p<0.01$

Any differences in the content of leukocytes in the blood of the rats between the groups were not found. The differences were stated in the content of lymphocytes. From table 7 it is followed that in all groups, excluding group 4, the rats receiving milk, had the high level of lymphocytes; it was signifcantly higher as compared to the rats which were fed the synthetic diet (group 6). It may indicate the favourable effect of active substances, contained in the milk, on immunological system. We may suppose that the main role is played, in this case, by lactoferrin, synthesized by epithelium of mammary glands and also, other peptides of whey proteins: alfa-lactoalbumins and peptides, coming from casein proteins: alpha- and beta-caseins which affect the blastogenesis of lymphocytes (Schanbacher et al., acc. Bernatowicz and Reklewska [1]).

In table 8 phagocytic activity of neutrophils and monocytes in peripheral blood of the rats and the mean intensity of fuorescence of neutrophils and monocytes, are given.

Table 8
Phagocytic activity of neutrophils and monocytes in peripheral blood of the rats

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
Phagocytic neutrophils (%)	56.89	52.73	57.63	58.30	59.47	47.84	16.95	0.97
Mean intensity of fuorescence of neutrophils (FU*)	607[a]	470[b]	503[ab]	610[a]	548[ab]	491[ab]	76.14	0.017
Phagocytic monocytes (%)	20.21	25.17	29.70	23.20	20.42	26.35	13.76	0.96
Mean intensity of fuorescence of monocytes (FU*)	323	273	352	371	327	327	86.88	0.85

*FU – unit of fuorescence (log)
Mean values of indicators in the rows, marked with pairs of letters differ statistically at $p \leq 0.05$

Any differences in respect of percentage content of monocytes and phagocytic neutrophils in the rats' blood between the particular groups were not found. On the other hand, the differences in the mean fuorescent activity of neutrophils between the groups of rats were observed; the differences in the mean activity of monocytes in the blood of the rats were not recorded (tab. 8). The rats of group 2, receiving the milk from the cows fed the diet with the addition of *Echinacea purpurea* were characterized by the lowest value of mean fuorescence of neutrophils. On the other hand, neutrophils in the group of the rats, receiving the milk with the additive of *Echinacea purpurea* (group 4) and in group 1, receiving the milk form the cows fed the diet with the addition of Herbatan revealed decisively better fuorescence properties as compared to the animals of the remaining groups.

Goel et al. [17] studied the effect of the particular groups of active compounds derived from *Echinacea purpurea* on the capability of macrophages to phagocytosis. The influence on stimulation of phagocytosis process was found only in case of the isolated fraction of alkamides. In the experiment, conducted by Cundell et al. [9] in the group of the rats, receiving extract of *Echinacea purpurea*, a low level of phagocytic activity of neutrophils and monocytes was maintained during the whole period of the experiment. The authors suggest that such result could be caused by the application of overground parts of plant, which in comparison with the root, contains smaller quantities of biologically active components (alkamides, polysaccharides, flavonoids). In the own studies, extract of *Echinacea purpurea* was obtained from overground part of the plant.

In table 9 value of IL-2 in blood serum of the rats according of the employed diet has been given.

Table 9
The content of IL-2 in blood serum of the rats

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
IL-2 ($pg \cdot ml^{-1}$)	204.00	194.53	254.77	263.00	214.52	200.43	78.65	0.25

Any statistically signifcant difference in the content of Interleukin 2 between the groups of the rats was not found but the rats from group 4, receiving the addition of *Echinacea purpurea* directly to the milk, were characterized by the highest numerical value. In the studies conducted with the rats, Goel et al. [17] did not state any signifcant effect of active substances, contained in *Echinacea purpurea*, on the content of IL2 in blood serum of the rats. The most important processes which are induced by Interleukine-2 include: proliferation of T lymphocytes, differentiation of T lymphocytes toward T cytocyclic lymphocytes, proliferation and differentiation of B lymphocytes, activation and proliferation of NK cells (the main group of cells of immunological system, being responsible for phenomenon of natural cytotoxicity).

Concentration of IL-4 did not exceed the threshold of determinability by ELISA test, as specified for 5 pg/ml. The IL-4 stimulates proliferation of B lymphocytes, induced by specific antigen and, in some cases, it may activate resting B lymphocytes. It is one of the most important factors which induce production of IgE by B lymphocytes.

Table 10 presents level of immunoglobulins in blood serum of the rats.

Table 10
The level of immunoglobulins in blood serum of the rats

Examined parameter	Diet of rats						SEM	p-value
	Milk from the cows receiving herbal extract in their ration		Milk with the direct addition of herbal extracts		Milk without the addition of herbal extracts	Synthetic		
	Herbatan	*Echinacea purpurea*	Herbatan	*Echinacea purpurea*				
	group 1	group 2	group 3	group 4	group 5	group 6		
IgG (g·l-1)	3.27	2.19	2.25	2.20	2.23	2.12	1.78	0.60
IgM (g·l-1)	0.48	0.51	0.66	0.56	0.48	0.70	0.21	0.38
IgE (g·l-1)	22.13	26.20	16.65	17.67	20.63	18.41	1.34	0.20

In spite of the highest level of IgG (the main antibody generated during the secondary response of organism), any statistically significant differences in blood serum of the rats of group 1 receiving milk from the cows fed the diet with the addition of 150 ml of Herbatan as compared to group 6, fed the synthetic diet, to group 5, receiving the milk without herbal additives and the rats from the groups which received the milk from the cows fed the diet with the additive of Herbatan and *Echinacea purpurea* (group 2) and from the groups where the additive of Herbatan and *Echinacea purpurea* extracts was employed directly to the milk (group 3 and 4), were not found. Any significant differences in the content of immunoglobulins: IgM, which were first synthesized at the present of antigen and IgE, the main pathogenic antibody connected with allergy, in the blood serum of the rats between the particular groups were not recorded, as well. Sokolnicka et al. [40] employed the preparations with *Echinacea purpurea* in their studies with the rats and did not obtain any differences in production of antibodies, in relation to control groups, in the contrary to the parameters of cellular immunity system.

The effect of herbal extract on functional value of milk was manifested by the lowest cholesterol and LDL content in the rats fed the milk diet with direct addition of Herbatan and *Echinacea purpurea* to milk, the highest level of erythrocytes and hemoglobin in case of the rats receiving the milk from cows fed the diet with Herbatan addition, by the intensive fluorescence activity of phagocytic neutrophils in the group of the rats receiving the milk with the addition of Herbatan in the diet, and in the group of the rats receiving the addition of *Echinacea purpurea* directly to the milk.

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