Journal of Animal and Feed Sciences, 6, 1997, 269–279

Herb as agents affecting the immunological status and growth of piglets weaned with body weight deficiency

R. Kołacz[1], Ewa Bodak[1], M. Świtała[2] and P. Gajewczyk[3]

[1] *Department of Animal Hygiene and Livestock Environment*
[2] *Department of Pharmacology and Toxicology*
[3] *Department of Swine Breeding and Production*
Agricultural University of Wrocław
Dikstеina 3, 51-617 Wrocław, Poland

(Received 16 July 1997; accepted 15 May 1997)

ABSTRACT

Immune deficiency was found in weaned pigs suffering from runting-stunting syndrome. It was manifested as decreases in: percentage of T-lymphocytes, phagocytic activity of neutrophils, the number of neutrophils capable of phagocyting and reducing nitrotetrazolium blue (NBT), DTH skin reaction to 1-chloro-3,4-dinitrobenzene (DNCB). The runt pigs were treated with: a mixture of 5 herbs (Chamomile flowers, Calendula florets, Fennel fruits, Fenugreek seeds, Sweet Basil herbs) and Biostymina – an aqueous aloe extract, given separately or together with the herb mixture. Administration of the five-herb mixture and/or Biostymina resulted in enhancement of neutrophil activity. Restoration of the T-lymphocyte population was observed only in the group treated with both preparations. The 5-herb mixture was the most effective in promoting weight gain compensation.

KEY WORDS: growing pigs, runting-stunting syndrome, immune response, growth, herb preparations

INTRODUCTION

The runting-stunting syndrome affecting suckling piglets develops into a serious production problem. The body weight deficiency at weaning later increases even two or three times during fattening as compared with healthy pigs of the same age (Rekiel, 1992).

Runting-stunting syndrome develops together with immune deficiency (Hennessy, 1987; Mysłowski, 1990; Świtała et al., 1994; Kołacz et al., 1994, 1995; Bodak-Koszałka et al., 1996), hence the pigs affected with the syndrome are more likely to suffer from infectious diseases in their severe or chronic form. The immune response to the infectious agent is only partial. This increases the risk of the pathogens carrier introducing infections of an epizootiologic character.

It has been assumed in this work that administration of herb preparations enhancing the immune response and normalizing the digestive function, to pigs with body weight deficiency may promote restoration of the immune response and body weight compensation.

The aim of the study was to determine the immune response and growth after treatment with herb preparations of pigs that were underweight at weaning.

MATERIAL AND METHODS

The study was carried during out 42 days on a commercial pig farm. The piglets weaned at 28 th day of life were divided into four groups, with 12 runt pigs (4-5 kg body weight) in each. The body weight deficiency at weaning was a result of low body weight at birth and disorders in lactating sows. Litters in which diarrhea occurred prior to weaning were excluded from the study. The results of earlier studies show that colibacteriosis markedly reduces the immune response (Świtała et al., 1997).

The four study groups were denoted as G-C group (negative control), and 3 experimental groups: G-H, G-B and G-HB in which herb preparations were administered. In addition, the G-N group contained 12 normal pigs weighing 7-8 kg.

In group G-H, the five-herb mixture was composed of the following dry herbs (Herbapol, Poland): Chamomile flowers (*Anthodium chamomillae*), calendula florets (*Flos calendulae*), fennel fruits (*Fructus foeniculi*), Fenugreek seeds (*Semen foenugraeci*), Sweet basil herbs (*Herba basilici*) in equal parts by weight. During 42 consecutive days of the experiment, herbs in an amount of 1.5 g/kg BW were infused and diluted with water at a proportion of 1:20. A fresh portion was poured into plastic containers every day, to which the animals had *ad libitum* access.

In group G-B group, an aqueous aloe extract under the trade name of Biostymina (Herbapol, Poland) was administered orally at a dose of 4 ml on days 1, 14 and 12 of the experiment.

In group G-HB group, both the herbal decoction and Biostymina were administered as described for groups G-H and G-B.

Each group of pigs was kept in a separate pen with free access to water and feed (PP-prestarter mixtures). The feeding value of the PP-prestarter mixtures was: ME, 13.5 MJ/kg; dry matter, 90%; crude protein, 23.2%; crude fat, 2.8%; crude fibre, 3.3%.

On the last day of experiment (day 42) venous blood was collected for the following tests: leukogram; phagocytic activity of neutrophils *ex vivo* (a) with the use of the nitrotetrazolium blue (NBT) reduction test according to Park et al. (1968) and (b) with the use of the *Staphylococcus epidermidis* phagocytosis test; percentage of T-Lymphocytes forming E-rosettes according to Binns (1978); the percentage of B-lymphocytes forming EAC rosettes according to Bianco et al. (1970); the IgG level with the use of radial immunodiffusion (RID kit, The Binding Site, U.K.); delayed type hypersensitivity (DTH) reaction to 1-chloro-3,4-dinitrobenzene (DNCB) in a skin test.

In the test of *S. epidermidis* phagocytosis, freshly collected blood (0.5 ml) was mixed with a suspension (0.35 ml) of live bacteria (density $3x10^8$ cells/ml) opsonized previously with swine blood serum and incubated at 37^0C for 30 min. The smears were stained using the MGG method and read under immersion. The percentage of phagocyting neutrophils and phagocyte index (the number of phagocytized bacteria per one phagocyting cell) were determined.

In skin test with DNCB 0.2 ml of 2% DNCB was used epidermically to sensitize the pigs on day 42 of the experiment. A skin reaction (under the ear) was evoked by the same dose of DNCB 14 days later and measured after 24 h.

The pigs were weighed twice, on the first and last day of the experiment.

The data obtained in the study were analysed statistically using a Student's t-test. Differences with $P<0.05$ were considered significant.

RESULTS

A comparison of the immune response of runt-untreated pigs with normal pigs is presented in Table 1. No marked differences in the percentage of blood neutrophils or lymphocytes in G-N and G-C pigs were found. However, a significant, 37% ($P<0.05$), decrease in NBT-positive cells and an 11% ($P<0.05$) decrease in neutrophils phagocyting *S. epidermidis* were observed in G-C pigs.

In G-C pigs the percentage of E-rosette forming T-lymphocytes was 32% lower ($P<0.05$), whereas the percentage of B-cells forming EAC-rosette was similar to that in G-N pigs. In the runt-untreated pigs (G-C) 20% lower (NS) level of IgG and 30% decreased DTH reaction in the skin test with DNCB ($P<0.05$) were found.

TABLE 1

Immunological indices in normal (n = 12) and runt (n = 10) pigs

Immunological indices	Normal pigs		Runt pigs	
	$\bar{x}$	± SD	$\bar{x}$	± SD
NBT-positive neutrophils, %	12.8	2.1	8.1	2.5*
Phagocyting neutrophils, %	58.3	5.7	52.0	5.8*
Phagocytic index	8.8	0.9	8.6	0.5
T-lymphocytes as E-rosettes, %	43.5	4.9	29.6	7.8*
B-lymphocytes as EAC-rosettes, %	42.9	6.2	43.6	4.4
IgG, mg/ml	13.5	3.6	10.8	3.4
DTH skin reaction to DNCB, mm	5.0	1.6	3.5	1.4*

* – $P < 0.05$

Response of runt pigs to herbaceous preparations

In comparison to G-C pigs, the following increase in neutrophils was observed in treated groups (Table 2): in G-H, 63% ($P < 0.05$), in G-B, 77% ($P < 0.01$), and in G-HB, 104% ($P < 0.05$). Besides, an increase in neutrophils phagocytic activity occurred in those groups (Figure 1). The percentage of NBT-positive cells increased in group G-H by 123%, in G-B by 156%, and in G-HB by 130% ($P < 0.05$). The percentage of neutrophils phagocyting *S. epidermidis* increased in group G-H by 36%, in G-B by 33%, and in G-HB by 26% ($P < 0.05$). The phagocytic index increased in group G-H by 12%, in G-B by 16% and in G-HB by 43% ($P < 0.05$) which proves the synergetic action of the herbs mixture and Biostymina.

The population of lymphocytes in pigs was not significantly changed after treatment with herb preparations (Table 2). Administration of the herb decoction or aloe extract increased the percentage of B-cells forming EAC rosettes (G-H 39%, G-B 50%, $P < 0.001$). However, in both G-H and G-B pigs

TABLE 2

Number of white blood cells (106/ml) in runt pigs untreated or treated with herbaceous preparation

Group	n	Leukocytes		Neutrophils		Lymphocytes	
		$\bar{x}$	± SD	$\bar{x}$	± SD	$\bar{x}$	± SD
Runt pigs							
Control (G-C)	10	8.5	1.8	2.2	1.0	6.2	1.8
Herbs (G-H)	10	11.5	1.5	3.6	0.6*	7.6	1.2
Biostymina (G-B)	10	10.2	2.4	3.9	0.7*	6.3	1.5
Herbs + Biostymina (G-HB)	10	12.0	3.6	4.5	1.3*	7.4	1.9
Normal pigs (G-N)	12	8.2	1.5	3.0	0.6	5.2	1.2

* – $P < 0.05$

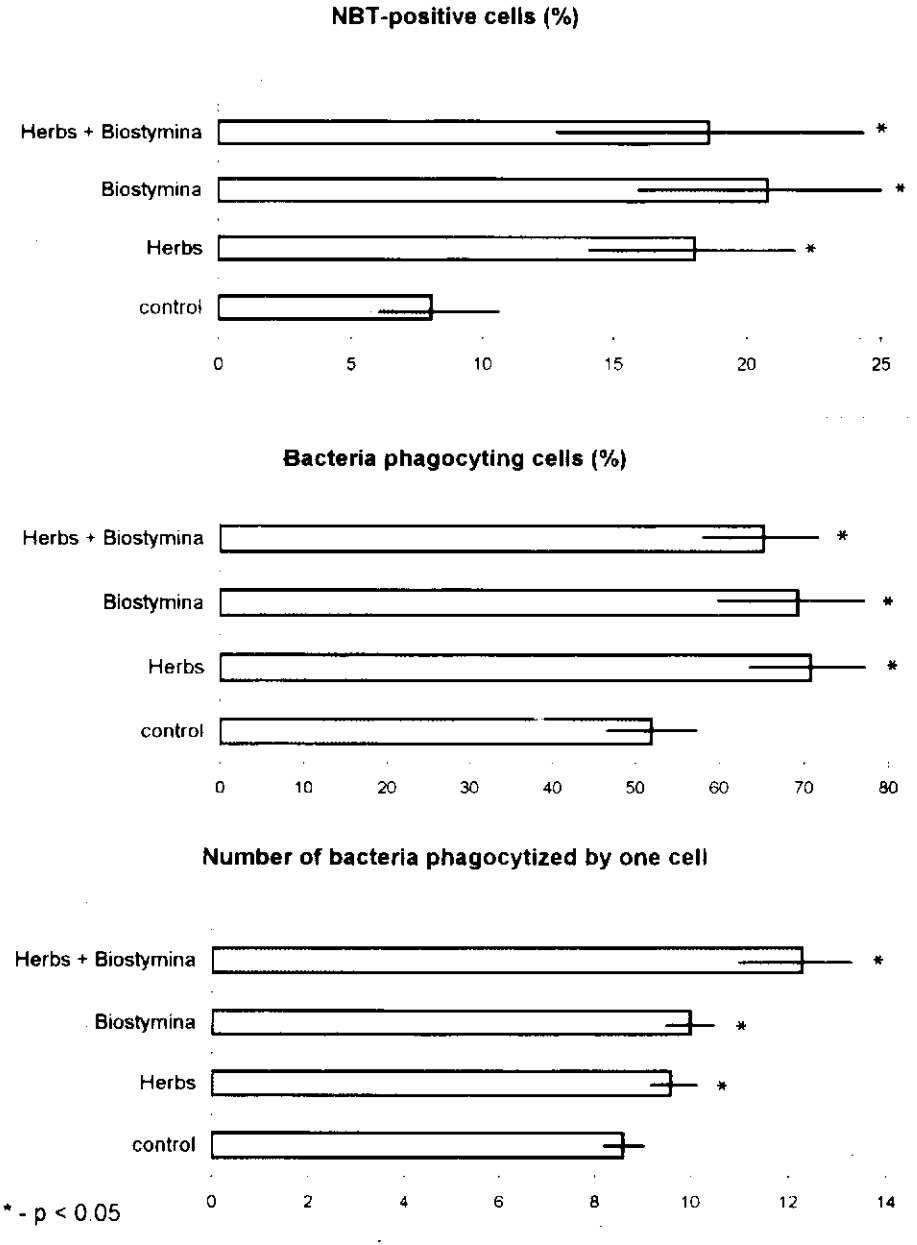

Figure 1. Phagocytic activity of neutrophils in runt pigs (n = 10 in each group)

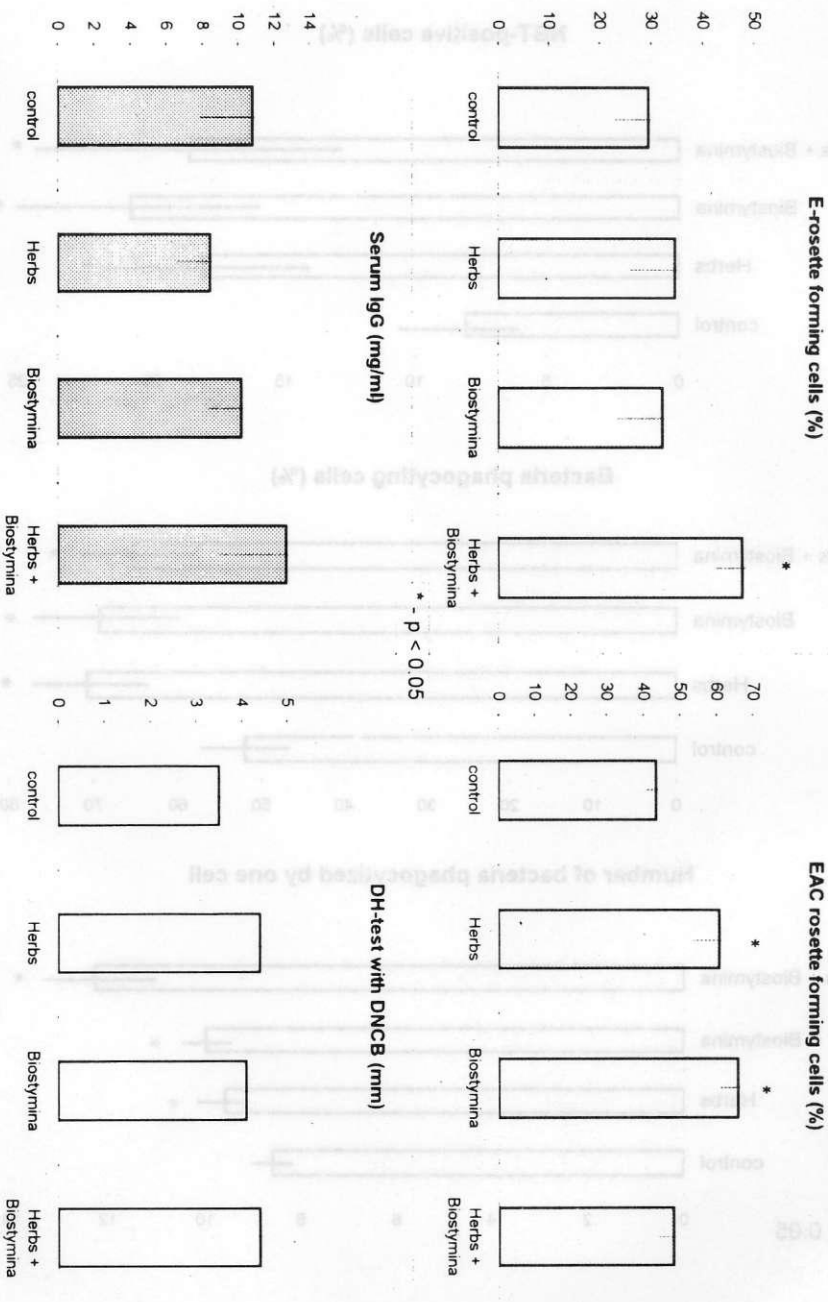

Figure 2. Immunological indices in runt pigs (n = 10 in each group)

there was no significant change in T-subpopulation forming E-rosettes (Figure 2). The opposite picture was seen in group G-HB, in which a significant increase in the percentage of E-rosette forming cells of 61% ($P < 0.05$), i.e., the level similar to that of G-N pigs, which occurred together with slight stimulation of EAC rosette formation.

The IgG level in the blood of G-H, G-B and G-HB pigs was not markedly different from that of the G-C group. In G-H pigs the mean IgG level was the lowest, while in G-HB pigs it reached the highest value.

In treated pigs (G-H, G-B and G-HB) the delayed-type hypersensitivity reaction to DNCB was only slightly higher than in G-C group (NS).

Growth of pigs

The initial body weight of entire runt population (G-C, G-H, G-B, G-HB) averaged 4.69 kg and was 2.43 kg (34%) lower than the weight of C-N pigs (Table 3). On the last day of the experiment the difference between G-C and G-N was 5.3 kg (29.6%).

Average daily gain (ADG) of G-N pigs was 257 g, while that of G-C 186 g ($P < 0.01$). The ADG of treated runt pigs was were as follows: G- H 232 g, G-B 201 g, G-HB 194 g.

On last day of the experiment the pigs taking the herbaceous decoction (G-H) showed the highest body weight of the runt pig groups (G-C, G-B, G-HB). The difference in body weight in 70-day-old pigs between G-C and G-H groups was 1.49 kg ($P < 0.05$). It is worth noting that the difference in ADG between runt pigs fed on herbs (G-H) and normal animals (G-N) was negligible (25 g). In the remaining groups (G-B and G-HB) the final body weight was not markedly different from that of G-C pigs.

TABLE 3

Body weight, average daily gain (ADG) and survival rate in runt pigs untreated or treated with herb preparations, and in normal pigs

Group	n	Body weight, kg				ADG g	Survival rate, %
		28 day		70 day			
		$\bar{x}$	± SD	$\bar{x}$	± SD		
Runt pigs							
Control (G-C)	12	4.81	0.71	12.62	1.26	186	83
Herbs (G-H)	12	4.37	0.38	14.11	1.14*	232	100
Biostymina (G-B)	12	4.88	0.47	13.32	1.07	201	100
Herbs + Biostymina(G-HB)	12	4.71	0.39	12.86	1.47	194	100
Normal pigs (G-N)	12	7.12	1.11*	17.92	1.37*	257	100

* – $P < 0.05$

DISCUSSION

The results confirm, that when the body weight deficiency occurs in young pigs then immune deficiency occurs as well. It is expressed by decreased neutrophils phagocytic activity, a decrease in the number of T-lymphocytes and a decrease in DTH skin reaction to DNCB. These findings corroborate our earlier results (Świtała et al., 1994; Kołacz at al., 1994; Bodak-Koszałka et al., 1996).

As is generally known, runting-stunting syndrome develops in young pigs on the background of under- or malnutrition. The latter leads to alimentary hypoproteinemia causing a reduction in lymphatic organs, followed by following their degenerative structural and functional changes, finally impairing phagocyting cell activity and antibody synthesis (Rzedzicki and Kowalska, 1992). Immune deficiency results in numerous infections, in the course of which one can observe stimulation of T-suppressor or temporal restriction of T-helpers activity. This leads to a decrease in immune system reactivity, obviously exposing the pigs to infections of secondary or mixed type (Pejsak and Truszczyński, 1993).

The five-herb mixture administered in the present study (G-H) proved to be immunostimulating. It seems possible that substances in Calendula florets were active in potentiating the cellular response in pigs. This herb is known to enhance defensive reactions, which makes it especially useful in the treatment of neoplastic diseases (Ożarowski, 1982). Chamomile flowers containing azulenes stimulating phagocytosis may have been directly involved in immune reactivity. The herbs could indirectly affect hormone and vegetative systems. For instance, Chamomile influences the function of the thyroid gland (Ożarowski, 1982). A correlation between thyroid function and a thymus-dependent response has been documented (Ślebodziński, 1993). Moreover, some substances contained in herbs (oils, silicon, calcium-phosphorus compounds) may constitute the kind of natural adjiuvants which can significantly enhance the response to antigens (Truszczyński and Pejsak, 1993).

Obviously, the mode of action of compounds entering into the originally composed mixture cannot be only univocally attributed to a chosen group of chemical substances present in those herbs. It is a fact that some of the compounds found in Fenugreek seeds, Chamomile flowers and Sweet Basil herbs may act on intestinal nutrient absorption and regulate intestinale peristalsis, whereas compounds isolated from Calendula florets and Fennel fruits stimulate secretion within the alimentary tract. Similarly, through their bactericidal action, Chamomile flowers and Sweet Basil herbs inhibit excessive growth of intestinal microflora, thus counteracting inflammation. These herbs appear to be stimulating regeneration processes on the intestinal membrane after past gastric-intestinal diseases.

The nutritive value of the herbs was of no less importance, especially as the herbs it included Fenugreek seeds which contain, among others, 20% of protein, 6-10% lipids, 2-6% mineral salts, carbohydrates, as well as vitamins A and D_3 in amounts comparable to cod-liver oil (Muszyński, 1958; Ożarowski, 1982). A mixture of herbs containing substances which regulate digestion and nutrient absorption affected the growth of G-H pigs, which was finally not much different from that in G-N pigs. Gajewczyk and Akińcza (1988) reported better feed intake and a 12% increase in daily gain after addition of a post-extractive infusion from Calendula florets and Chamomile flowers, a by-product of the pharmaceutical industry. Other authors (Mazurczak et al., 1973; Gajęcki, 1988; Maciołek, 1992) have found a more or less spectacular influence of herbs on daily gain of pigs. A positive impact on growth was atributed to the improvement of feed flavour, hence its better intake, as well as to the improvement of health and vitality. It could also be supposed that some herbs have an anti-stress effect, probably due to their content of sedative substances.

An immunostimulating reaction was expected after administration of aloe extract (Biostymina), whose immunotropic effects have been well documented (Ożarowski, 1982; Furowicz, 1989; Furowicz et al., 1990). The increase in phagocytic activity of neutrophils in G-B pigs resulted from the known properties of this preparation, such as intensified pathogenic cell absorption and increased granulopoesis. In increase in the lymphocytes B subpopulation forming EAC rosettes was also observed in the G-B pigs, while an increase in the IgG level was not reported.

In G-B pigs body weight gains were not markedly changed. Other studies carried out on young pigs (11-12 weeks) suffering from runting syndrome proved that after three doses of Biostymina of 2 ml/kg BW per dose, daily gain worsened by 13% and feed intake decreased by about 10% in comparison to the untreated pigs (Rekiel, 1992). These results suggest an undesirable effect of Biostymina on the growth of young pigs. The reason for such a reaction is not clear. In calves (4-5 months old) suffering from a chronic form of *Bronchopneumonia enzootica*, the immunostimulating of Biostymina such as significant increase in phagocytic reactions and serum IgG level was accompanied by its stimulating influence on daily gain (Furowicz, 1989; Furowicz et al., 1990).

Administration of the herbs mixture and Biostymina (G-HB) significantly affected the immune response of runt pigs. Besides the stimulating effect on the percentage of neutrophils phagocyting *S. epidermidis*, synergism of action of preparations on the number of phagocytized bacteria by those cells was not reported when those preparations were administered separately. However, the number of B lymphocytes forming EAC rosettes did not rise. A worse weight gain than that in the group taking the herb mixture alone was found (G-H),

which suggests that Biostymina overcomes the stimulating influence of herbs on gain and inhibits the growth of pigs.

CONCLUSIONS

Young pigs with underweight at weaning were proved to suffer from immune deficiency manifested as a decrease in neutrophil phagocytic activity, in the number of T-lymphocytes in peripheral blood and in the DTH reaction to DNBC.

Administration of a five-herb to the form of a decoction diluted with drinking water, or Biostymina, an aqueous aloe extract, or both preparations together to those runt pigs appeared to restore the immunei reaction.

The five-herbs mixture, probably through its regulating action on digestive processes, was the most effective for weight gain compensation.

Biostymina did not restore weight gain of runt piglets.

REFERENCES

Bianco C., Patrick R., Nussenzweig U., 1970. A population of lymphocytes bearing a membrane factor for antigen-antibody-complement complexes. Separation and characterization. J. Exp. Med. 132, 702-720

Binns R.M., 1978. Sheep erythrocytes in pigs, cattle and goats demonstrated in the presence of dextran. J. Immun. Met. 21, 197-210

Bodak-Koszałka E., Świtała M., Kołacz R., 1996. Immunological reactivity of porkers with body weight defciency after levamisole and other immunomodulators administration (in Polish). Acta Acad. Agric. Techn. Olst., Veterinaria 23, 161-172

Furowicz A., 1989. Biological immunomodulators in veterinary prophylaxis and therapy (in Polish). Prz. hod. 57 (8), 28-30

Furowicz A., Lewandowska S., Czernomysy-Furowicz D., Łoczewski P., Grupiński T., 1990. Biostymina in calf bronchopneumonia therapy and prophylaxis (in Polish). Nowiny wet. 20 (2), 113-123

Gajewczyk P., Akińcza J., 1988. Usage of herb by-products from pharmaceutical industry in pig feeding prophylaxis (in Polish). Prz. hod. 56 (16), 23-29

Gajęcki M., 1988. Prophylactic use of herbal preparations for sows about the parturition period (in Polish). Acta Acad. Agric. Techn. Olst., Veterinaria, 17, Suppl. A , 1-55

Hennessy K.J., Blecha F., Pollmann D.S., Kluber E.F., 1987. Isoprinosine and levamisole immunomodulation in artificially reared neonatal pigs. Amer. J. Vet. Res. 48, 477-480

Kołacz R., Świtała M., Bodak-Koszałka E., Obmińska-Domoradzka B., Dębowy J., 1994. Levamisole and mechlorctamine in immunostimulating doses and herbs as immunorestorative agents for weaned pigs with runting syndrome. Proceedings of VIII International Congress of Animal Hygiene, St. Paul, Minnesota, USA, pp. 105-108

Maciołek H., 1993. Herb's mix for sows using in farrowing period prophylaxis (in Polish). Trzoda chl. 31 (8-9), 30-32

Markiewicz K., Szeligowski E., 1979. Prevention and prophylaxy of noninfectious diseases in industrialized livestock farming (in Polish). Med. wet. 35 (2), 92-96

Mazurczak J., Kotowski K., Pieniążek J., 1973. The role of herborization in the prophylaxy and treatment of alimentary tract disturbances in piglets (in Polish). Med. wet. 29 (6), 329-332

Muszyński J., 1958. Phytotherapy and Plant-derived Drugs (in Polish). PZWL, Warszawa

Mysłowski P.,1990. The prophylactic use of immunomodulators : imutiol (DTC) or levamisole in swine livestock farming. Ph Thesis. Wrocław Agricultural University

Ożarowski A.,1982. Phytotherapy (in Polish). PZWL, Warszawa

Park B.H., Firkig S., Smithwick E.M., 1968. Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet 2, 532-534

Pejsak Z., Truszczyński M., 1993. Fundamentals of immunoprophylaxis of infectious diseases of swine (in Polish). Med. wet. 49 (2), 55-58

Rekiel A., 1992. Administration of Biostymina in rearing of runt piglets (in Polish). Trzoda chl. 30 (6), 6-7

Rzedzicki J., Kowalska M., 1992. The influene of nutrition on immunity of animals (in Polish). Med. wet., 48 (6), 249-252

Ślebodziński A. B., 1991. Feedback mechanisms and interaction between hormones and immune system (in Polish). Med. wet. 47 (7), 289-293

Świtała M., Obmińska-Domoradzka B., Kołacz R., Dębowy J., 1994. The influence of levamisole and mechlorethamine in immunostimulating doses on the immune system of runt pig. Pol. J. Immun.19, suppl. 2, 80-81

Świtała M., Kołacz R., Bodak-Koszałka E., Gajewczyk P., 1997. Hematological and biochemical parameters of blood and immune response of runt weaners. Arch. Vet. Pol. (in press)

Truszczyński M., Pejsak Z., 1993. Types of adjuvans and their capabilities of stimulating the immune system (in Polish). Med. wet. 49 (11), 486-493

STRESZCZENIE

Zioła jako środki wpływające na układ immunologiczny i wzrost prosiąt odsadzonych z niedoborem wagi ciała

U odsadzonych warchlaków z objawami charłactwa wykazano niedomogę układu odpornościowego. Wyrażała się ona: spadkiem odsetku limfocytów T, obniżeniem aktywności neutrofili (zdolnych fagocytozy bakterii), III spadkiem liczby neutrofilów zdolnych do fagocytozy i redukcji błękitu nitro-tetrazoliowego (NBT), zmniejszeniem skórnej reakcji nadwrażliwości późnego typu (DH) na 1-chloro-3,4- dinitrobenzen (DNCB). U świń charłaczych zastosowano następujące preparaty ziołowe: napar mieszanki złożonej z 5 ziół – kwiatostanu rumianku, kwiatu nagietka, owocu kopru, nasion kozieradki i ziela bazylii oraz biostyminę – wodny wyciąg z aloesu. Preparaty podawano osobno i w połączeniu. Obydwa preparaty podawane osobno lub razem wywołały wzrost aktywności fagocytarnej neutrofilów. Odnowę subpopulacji T limfocytów obserwowano tylko w grupie, w której podawano oba preparaty. Po podaniu mieszanki z pięciu ziół przyrost masy ciała zwierząt był najlepszy.