

## Characteristics of Selected Peripheral Blood Parameters in Polar Fox (*Alopex lagopus* L.) Fed Diets with Inulin

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This study aimed at investigating changes in selected peripheral blood parameters in male polar foxes fed diets with different supplementation of inulin: 0.25% (group E1), 0.5% (E2) and 1% (E3). The blood for analysis was sampled from the brachial vein. The study showed that adding 0.25 and 0.5% of inulin to fox feed resulted in a lower content of haemoglobin (Hb) as well as mean mass of Hb in red blood cells in the 0.5% inulin group. The total count of thrombocytes decreased significantly with a higher level of prebiotic, while the total number of white blood cells and the percentage of different leukocytes tested remained invariable. The lowest supplementation of inulin affected the partial pressure of carbon dioxide, however, the remaining acid-base parameters did not change. The present study provides the first preliminary information about the effect of dietary inulin on some haematological indices and acid-base parameters in adult polar foxes. The results may be helpful in practice to improve the health condition of farmed polar foxes.

Key words: Polar fox, haematological indices, acid-base balance, inulin.

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Inulin is a natural storage material accumulated in some plants such as garlic, onion, chicory, Jerusalem artichoke, artichoke and asparagus (HESTA *et al.* 2006; KOLIDA & GIBSON 2007). This fructan is a polysaccharide composed of a few dozen fructose molecules connected to one another with  $\beta$ -2,1 bonds. This bond type determines the prebiotic properties of this substance (SWANSON *et al.* 2002a). Inulin is not digested by alimentary tract enzymes, however, it undergoes degradation only when affected by bacterial microbiota of the large intestine. Its positive effect on the host is a consequence of the stimulation of the development of beneficial intestinal microbiota, for example *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in human and rat intestine and, at the same time, the inhibition of harmful bacterial colonization, mostly *Escherichia coli*, *Enterobacterium* or *Bacteroides* (KAUR & GUPTA 2002; JÓZEFIK *et al.* 2005). The main functions of microorganisms in the alimentary tract include promoting digestive processes and ileal peristalsis, and the synthesis of

some vitamins and metabolites that exert antibacterial activity limiting ileal colonization with pathogenic bacterial strains (MURRAY *et al.* 2005). Inulin fermentation in the large intestine results in the production of lactic acid and short-chain fatty acids (SCFA). Their ileal concentration increases as a response to the introduction of prebiotics to the ration (PROPST *et al.* 2003; VERLINDEN *et al.* 2006; MIDDELBOSS *et al.* 2007). Both lactic acid and SCFA are mostly produced in the first part of the large intestine – the ascending colon, thus locally supplying energy to epithelial cells. Short-chain fatty acids together with lactic acid decrease digesta pH creating a less favourable environment for pathogens (CAMPBELL *et al.* 1997; SWANSON *et al.* 2002a; GALISTEO *et al.* 2008). Numerous studies performed in various animal species (e.g. dog, cat and rat) reported a positive effect of prebiotic fructans on carbohydrate, lipid and protein metabolism and increased bioavailability of minerals (VERDONK *et al.* 2005; HESTA *et al.* 2006; LOBO *et al.* 2009). However, the effect of the fructans applied de-

depends on many factors including the type of the prebiotic, animal species, feed type (animal or plant feed), rearing conditions and dietary concentration (PROPST *et al.* 2003; VERDONK *et al.* 2005). An exemplary content of inulin used in experimental diets for different animal species was: pigs – 1.5% (FARNWORTH *et al.* 1992), dogs – 3% (VERLINDEN *et al.* 2006), poultry – 5% (CHAMBERS *et al.* 1997), rats – 10% (JÓZEFIAK *et al.* 2005). The beneficial biological activity of inulin was also confirmed in humans (GIBSON & ROBERFROID 1995; POOL-ZOBEL 2005; FOTIADIS *et al.* 2008).

Despite the fact that gastrointestinal diseases are considered to be the most frequent health problem in farmed polar fox, feeding experiments with inulin have not been conducted in this species so far. The most serious bacteriological threat in intensive production of carnivorous fur animals is *Salmonella* that causes gastrointestinal problems. This particularly concerns foxes which after infection may become carriers of this bacteria (most often: *S. typhimurium*, *S. choleraesuis*, *S. dublin* and *S. enteritidis*) (MALICKI & BINEK 2004). Potential sources of contamination on farms include faeces, contaminated feed, poor sanitary conditions on farms, as well as wild birds and rodents. Another bacteriological risk is *Escherichia coli* which is part of the physiological intestinal flora, but in adverse conditions becomes pathogenic. This is very dangerous even for healthy animals, as Colibacillosis may be an infectious disease, usually acute, with intense diarrhoea (GLIŃSKI & KOSTRO 2002; HANDELAND *et al.* 2008). The application of prebiotics could offer potentially satisfactory effects, mostly in the prophylaxis of alimentary canal disease.

Blood is a very sensitive indicator of metabolic changes in both physiological and pathological processes in the organism. The changes of all compounds introduced into the body impinge on the level of haematological parameters in a very short time. The results of the studies evaluating the effect of prebiotics on the level of selected blood indices are not unequivocal. The discrepancies reported by different authors in dogs, using different types of prebiotics and/or plants which are their natural source, concern especially white blood cells (SWANSON *et al.* 2002b; GRIESHOP *et al.* 2004). The investigations by VERLINDEN *et al.* (2006) on using inulin in a dog diet revealed a significant increase in the total number of leukocytes in the group of animals with 3% addition of this prebiotic.

The commencement of large-scale studies on the application of inulin in fox feeding based on analyses of morphological blood parameters, both red and white cells, seems to be fully justified. Due to the intense absorption of acid products produced in the process of inulin fermentation, into the blood-

stream, the study was extended to an assessment of acid-base balance indicators.

The aim of this research was to determine the effect of dietary inulin concentration on selected haematological parameters and acid-base balance in polar fox blood.

## Material and Methods

### Experimental animals

The study was conducted under a protocol approved by the Local Ethical Committee in Bydgoszcz (No. 2/2010 from the 21 January 2010).

The feeding experiment included twenty-four clinically healthy male polar foxes (*Alopex lagopus* L.) of similar body weight:  $9.15 \pm 0.15$  kg ( $\bar{x} \pm SD$ ). The foxes originated from a domestic reproductive farm and were examined in the Biological Testing Laboratory of the Department of Animal Physiology. The animals were individually weighed and randomly divided into four feeding groups, so that each group of foxes had a similar body weight distribution. The foxes were housed individually in metabolic cages located in a room with automatically controlled humidity (relative humidity 55%), temperature (12°C) and air exchange, with a 14h dark: 10h light cycle.

### Experimental diets

There were four dietary treatments: control (C) and three experimental setups (E1, E2, E3). During 16 days of the feeding experiment, the foxes from the control group were fed a standard diet based on animal offal and excreted cereals, supplemented with fiber preparation and a vitamin-mineral mixture. The composition and nutrient content in the standard diet is presented in Table 1. The diet complied with metabolisable energy (ME) requirements:  $376.8$  kJ ME kg<sup>-1</sup> of body weight (NRC 1982). Experimental diets varied in the amount of inulin (FRUTAFIT® IQ, ORAFTI, Belgium) incorporated into the diets instead of the fiber mixture (dried beet pulp). The content of the fiber mixture and inulin preparation in experimental diets was as follows: 17.5 and 2.5 (E1); 15.0 and 5.0 (E2); 10.0 and 10.0 g kg<sup>-1</sup> fresh matter (E3). All diets (standard and experimental) were prepared a few days before testing, homogenized, divided into daily rations for each fox and stored in feed cups at -25°C until use. Daily rations for feeding on the next day were taken out of the freezer and thawed at 5°C. The foxes were fed once a day at 8 a.m. and had free access to water.

Table 1  
Composition and nutrient content in  
standard diet fed to polar foxes

Components, g kg <sup>-1</sup> as fed	
Fish offal	400
Poultry offal	223
Meat meal (55%)	60
Excruded cereals	100
Fiber mixture <sup>1</sup>	20
Vitamin mineral mixture <sup>2</sup>	2
Water	195
Nutrients	
Dry matter (DM), g kg <sup>-1</sup> as fed	253
ME, kJ kg <sup>-1</sup> as fed	5025
Crude protein, g kg <sup>-1</sup> DM	368
Crude fat, g kg <sup>-1</sup> DM	142
N-free extractives <sup>3</sup> , g kg <sup>-1</sup> DM	345
Crude fibre, g kg <sup>-1</sup> DM	22
Ash, g kg <sup>-1</sup> DM	123

<sup>1</sup> dried beet pulp

<sup>2</sup> Guyofox Plus, concentration per 1 g: vit. A 3000 j.m.; D<sub>3</sub> 300 j.m.; E 50 mg; K 0.5 mg; B<sub>1</sub> 22 mg; B<sub>2</sub> 3 mg; B<sub>6</sub> 3 mg; B<sub>12</sub> 0.02 mg; H 0.03 mg; folic acid 0.3 mg; PP 5 mg; calcium panthotenate 3.15 mg; choline chloride 50 mg; Mn 7.5 mg; Zn 10 mg; organic Fe 20 mg; non-organic Fe 4.8 mg; Se 0.058 mg; Cu 1.25 mg; Co 0.01 mg

<sup>3</sup> by difference: N-free extractives = dry matter – (crude protein + crude fat + crude fiber + ash).

### Chemical analysis

Chemical analysis of the standard diet (dry matter, crude protein, crude fat, crude fiber and ash) were performed according to the methods of the Association of Official Analytical Chemists (AOAC 2010). On the last day of the experiment (at 7:00 a.m., prior to feeding) blood was collected from all the animals using brachial vein puncture and placed into two kinds of test tubes. The first one was a 4 ml tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The samples were analysed with an automated analyser for haematology, ADVIA 120 (Simens AG, Munich, Germany). The following haematological parameters were determined: red blood cell count (RBC), haematocrit index (Ht), haemoglobin concentration (Hb), white blood cell count (WBC) and the number of lymphocytes, granulocytes and monocytes as the percentage in the total number of WBC as well as blood platelets – thrombocytes (PLT). Red blood cell volume (MCV), mean mass of hae-

moglobin in a RBC (MCH) and mean haemoglobin concentration in a red blood cell (MCHC) were also determined. The second type of tubes used for blood collection consisted of 100  $\mu$ l heparinized capillaries. With the use of the AVL COMPACT 1 acid-base balance analyzer (Roche Diagnostic, Basel, Switzerland) the following acid-base equilibrium parameters were defined: pH, partial pressure of carbon dioxide (pCO<sub>2</sub>), partial pressure of oxygen (pO<sub>2</sub>), concentration of bicarbonate ions (HCO<sub>3</sub><sup>-</sup>act), total content of carbon dioxide (ctCO<sub>2</sub>), base excess or deficit (BE) and saturation of haemoglobin with oxygen (O<sub>2</sub> SAT).

### Statistical analysis

All results were expressed as means ( $\bar{x}$ ) with standard deviations (SD) and subjected to one-way analysis of variance using the Statistica 8.0 PL software (STATSOFT, INC. 2008). The *post hoc* Duncan test was applied. The probability of P<0.05 was accepted as significant.

### Results and Discussion

The content of selected haematological parameters of polar foxes fed diets with inulin is presented in Table 2.

Taking into consideration the role of blood in homeostasis-maintaining and easy blood sampling, blood tests are useful in determining whether the applied diets were adequate. In practice, it is important to reveal and interpret body condition anomalies. Regular observation of haematological indices can signal the threat of disease long before any symptom appears. In our study the count of red blood cells, the haematocrit value and haemoglobin concentration of experimental animals were within fox haematological reference values determined by BENN *et al.* (1986) as well as BERESTOV and BRANDT (1989). However, there were differences in the content of Hb in relation to the level of inulin in the diets. In comparison with the control group, the concentration of this parameter in the remaining groups was lower and in animals from E1 and E2 groups the haemoglobin content decreased significantly (P<0.05). The reason for these differences is unclear. The literature available does not report on any information on the haematological parameters of foxes in relation to inulin or other fructan supplementation. VERLINDEN *et al.* (2006) in an experiment on adult dogs fed a diet with inulin did not observe an influence of supplement on Hb concentration nor on the count of red blood cells and haematocrit index. The values of MCV in our study did not show any significant differences. However, MCH was lowest in animals from the E2 group fed a diet supplemented

Table 2

Haematological parameters of polar foxes fed diets with inulin ( $\bar{x} \pm SD$ )

Parameter	Group			
	C	E1	E2	E3
Red blood cells, $T l^{-1}$	$9.8 \pm 0.82$	$9.3 \pm 0.35$	$9.5 \pm 0.32$	$9.7 \pm 0.49$
Haematocrit, $l l^{-1}$	$0.56 \pm 0.03$	$0.52 \pm 0.02$	$0.53 \pm 0.04$	$0.55 \pm 0.03$
Haemoglobin, $g l^{-1}$	$184^a \pm 7.50$	$174^b \pm 4.83$	$171^b \pm 6.72$	$176^{ab} \pm 7.03$
MCV, fl	$56.8 \pm 1.89$	$56 \pm 0.63$	$55.5 \pm 3.00$	$57.2 \pm 3.07$
MCH, pg	$19^a \pm 0.83$	$19^{ab} \pm 0.32$	$18^b \pm 0.42$	$18^{ab} \pm 0.54$
MCHC, $g l^{-1}$	$330^{ab} \pm 6.86$	$335^a \pm 3.93$	$324.8^{ab} \pm 10.08$	$321^b \pm 9.85$
White blood cells, $G l^{-1}$	$7.46 \pm 1.63$	$7.68 \pm 1.89$	$7.4 \pm 0.58$	$7.4 \pm 0.78$
Lymphocytes, %	$39.40 \pm 11.72$	$37.3 \pm 9.35$	$45.20 \pm 11.82$	$45.0 \pm 7.72$
Granulocytes, %	$56.60 \pm 16.91$	$56.67 \pm 13.27$	$52.20 \pm 10.11$	$51.8 \pm 7.18$
Monocytes, %	$4.00 \pm 2.45$	$6.00 \pm 2.53$	$2.60 \pm 2.40$	$3.17 \pm 2.93$
Thrombocytes, $G l^{-1}$	$519.4^{ab} \pm 59.31$	$564.5^a \pm 57.55$	$459.8^b \pm 38.94$	$461.5^b \pm 79.53$

 $\bar{x}$  – mean value; SD – standard deviation

C – Control group (without inulin)

E1; E2 and E3 – Experimental groups (the content of inulin: 2.5; 5.0 and 10  $g kg^{-1}$  fresh matter, respectively)a,b – means in the row with different letters differ significantly ( $P < 0.05$ ).

with 0.5% of inulin. In comparison with the control group, the MCH value decreased significantly ( $P < 0.05$ ) which was closely connected with the lowest Hb concentration in the same group, presented above. On the other hand, the highest levels of mean haemoglobin concentration in red blood cells was found in foxes fed the lowest dose of inulin (0.25%). In the present study the MCHC concentration in group E1 differed significantly ( $P < 0.05$ ) in comparison with the E3 group fed the highest dose of prebiotic (1%) and reached levels of 335 and 321  $g l^{-1}$ , respectively.

The number of leukocytes in the blood of carnivorous animals is very unstable and changes affect both the total number of WBC and the percentages of each leukocyte type (BERESTOV & BRANDT 1989; MORITZ *et al.* 2004). The present study did not reveal significant inulin-dependent differences in the total number of WBC nor in the lymphocyte, granulocyte or monocyte content. The results of our study do not agree with the data reported by SWANSON *et al.* (2002b) who fed dogs diets with the addition of fructooligosaccharides – FOS (0.5%), mannanooligosaccharides – MOS (0.5%) or FOS + MOS (0.5% of each). Lymphocytes, expressed as a percentage of total WBC, significantly increased in the group with MOS compared to the control animals. GRIESHOP *et al.* (2004) evaluated the effects of prebiotics on nutritional and immunological characteristics in senior dogs. The study showed that MOS (1%) did not affect the WBC concentration, however, dietary

supplementation with chicory (1%) or MOS + chicory (1% of each) increased the neutrophil content, as compared to control dogs. The lymphocyte level, however, decreased in the animal diet supplemented with MOS and MOS + chicory.

The primary function of PLT concerns haemostasis, in the formation of clots in blood coagulation. Thrombocytes also mediate signal transmission between cells and influence immune responses. Abnormalities in the content of PLT in peripheral blood can result from conditions that affect the bone marrow and disturb the production of platelets from bone marrow stem cells (e.g. vitamin B<sub>12</sub> shortage, folate deficiency or liver damage). The present study showed differences in the number of PLT in the blood of experimental foxes. Higher ( $P < 0.05$ ) content of PLT was determined in the E1 group (564.5  $G l^{-1}$ ) in comparison with E2 and E3. PRZYSIECKI *et al.* (2010) showed no effect of the addition of herb preparation or yeast to the feed of growing foxes on haematological parameters including thrombocytes. In this study the number of PLT ranged from 380.80 to 591.33  $G l^{-1}$ . The total count of thrombocytes in our foxes compared with dogs fed an inulin-supplemented diet (VERLINDEN *et al.* 2006) was much higher (460-564  $G l^{-1}$  in foxes and 375-378  $G l^{-1}$  in dogs), however, these results were within the reference values determined by BENN *et al.* (1986).

Table 3 shows the content of acid-base balance parameters in polar fox blood. All indices tested

Table 3

Acid-base balance parameters in polar fox blood fed diets with inulin ( $\bar{x} \pm SD$ )

Parameters	Group			
	C	E1	E2	E3
pH	7.3 ± 0.04	7.3 ± 0.04	7.3 ± 0.01	7.3 ± 0.02
pCO <sub>2</sub> , kPa	6.8 <sup>a</sup> ± 0.96	5.1 <sup>b</sup> ± 0.23	6.9 <sup>a</sup> ± 0.86	6.3 <sup>ab</sup> ± 0.86
pO <sub>2</sub> , kPa	6.5 ± 0.86	6.2 ± 0.57	5.7 ± 0.88	6.79 ± 1.11
HCO <sub>3</sub> <sup>-</sup> , act mmol l <sup>-1</sup>	22.9 ± 1.68	19.3 ± 2.23	23.7 ± 3.04	22.5 ± 2.59
ct CO <sub>2</sub> , mmol l <sup>-1</sup>	24.5 ± 1.89	20.5 ± 2.29	25.2 ± 3.25	23.9 ± 2.76
BE, mmol l <sup>-1</sup>	- 4.73 ± 1.15	- 3.83 ± 1.67	- 3.85 ± 2.33	- 4.37 ± 1.85
O <sub>2</sub> SAT, %	78.4 ± 4.80	78.6 ± 5.51	71.0 ± 9.33	80.8 ± 7.17

 $\bar{x}$  – mean value; SD – standard deviation

C – Control group (without inulin)

E1; E2 and E3 – Experimental groups (the content of inulin: 2.5; 5.0 and 10 g kg<sup>-1</sup> fresh matter, respectively)

a,b – means in the row with different letters differ significantly (P&lt;0.05).

remained within the values noted by other authors in the venous blood of dogs (RUBASH 2001; NEMEC *et al.* 2003; POMIANOWSKI *et al.* 2004). As is well known, acid-base status in the organism may be influenced by anomalies in respiration inducing respiratory acidosis or alkalosis. All remaining anomalies are referred to as metabolic. Non-respiratory (metabolic) acidosis can result from different factors *e.g.* the formation of acids as a product of metabolism. According to the information given above, inulin and other prebiotics increase SCFA concentration and so cecal and faecal pH can change. Most *in vitro* and *in vivo* studies have reported that a decrease in large bowel pH was due to the greater production of SCFA in dogs after prebiotic supplementation (FLICKINGER *et al.* 2000; SWANSON *et al.* 2002b; FABER *et al.* 2011). However, in some of the studies reported, there were no significant changes in faecal pH following prebiotic supplementation (CAMPBELL *et al.* 1997; SWANSON *et al.* 2002b; VERLINDEN *et al.* 2006). A decreased intestinal pH by high lactate and SCFA concentration in the colon did not always result in the same change in faecal pH. These differences may be explained by SCFA absorption. Up to 95% of the acids produced are rapidly absorbed from the colon into the blood stream (HESTA *et al.* 2006). In the present experiment the supplementation of fox diets with inulin affected neither the pH nor any other remaining acid-base balance parameters of the peripheral blood, except for the pCO<sub>2</sub>. In comparison with the control and E2 groups, in the E1 group the concentration of pCO<sub>2</sub> was significantly lower (P<0.05), which was connected with the lowest total content of ctCO<sub>2</sub> and the lowest base deficit (BE) in animals fed a

diet supplemented with 0.25% of inulin. However, these differences were non-significant. Based on the literature available, it is difficult to explain and to relate any such changes to the level of inulin in the diets. According to the Henderson-Hasselbalch equation, the pCO<sub>2</sub> pressure is a measure of the respiratory component, causing changes in the plasma pH. The parameter of metabolic constituent is the concentration of HCO<sub>3</sub><sup>-</sup> act and, optionally, ctCO<sub>2</sub>, both of which present a sum of bicarbonate ion concentration and CO<sub>2</sub> dissolved in blood plasma. The HCO<sub>3</sub><sup>-</sup> level in blood is also determined by the content of O<sub>2</sub> SAT (SIGGAARD-ANDERSEN & FOGH-ANDERSEN 1995; CONSTABLE 2000).

In conclusion, the present study shows that addition of 0.25 and 0.5% inulin to polar fox diets resulted in a lower content of haemoglobin as well as mean mass of Hb in red blood cells (MCH) in the 0.5% inulin group. The total count of thrombocytes significantly decreases with a higher level of prebiotic while the total number of white blood cells and the percentage of the types of leukocytes tested remains invariable. The lowest supplementation of inulin affects the partial pressure of carbon dioxide, yet the remaining acid-base parameters do not change. Generally, we conclude that inulin supplementation does not have a negative influence on the peripheral blood indices tested. The present results, being the first of this type, can further enhance the development of our knowledge and facilitate the practical use of the results by providing preliminary information on the effects of dietary inulin on some haematological indices and acid-base balance parameters in adult polar foxes.

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