*Journal of Animal and Feed Sciences, 24, 2015, 226–234*
*The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna*

# The effect of early-life kidney bean lectin administration on pig performance in the peri-weaning period – a safety study

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**KEY WORDS:** kidney bean, lectin, phytohaemagglutinin, haematology, ALAT, microbiota, intestinal histopathology, piglets

Received: 14 April 2015
Revised: 20 August 2015
Accepted: 9 September 2015

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**ABSTRACT.** The present study aimed to assess the safety issues of supplying kidney bean lectin extract early in life to piglets. Four litters totalling or 30 piglets were used. On postnatal day (PD) 10–11, thirty piglets were divided into 3 experimental groups (T1, T2, and T3), each consisting of 10 animals. On PD 10 or 11, group T1 (control) received water. Groups T2 and T3 received, respectively, a dose of 640 (recommended) and 6400 haemagglutination/piglet (10 x recommended) of kidney bean extract orally in a water suspension. On PD 42, all of the pigs were euthanized, and 6 piglets from each group were randomly taken for necropsy, sampling tissue for histology and bacteriological analyses of the lower gut. Blood was withdrawn before euthanasia for haematology and biochemistry. There were no significant differences in mortality, feed intake or body weight gains among the groups. All examined blood parameters were within the physiological range. No significant effects of the two doses of kidney bean extract on tissue morphology were observed in the post mortem inspection and histology. No significant differences among the groups in the number of aerobic and anaerobic bacteria were found. Concluding, a one-month tolerance study with the recommended and 10-fold recommended dose of kidney bean extract revealed no negative effects on piglet health or performance.

## Introduction

In intensive pig production, early weaning is a key point in increasing sow productivity. On one hand, it increases the number of pigs delivered annually. On the other hand, it increases the intensity of digestive disorders due to discrepancies between the feed composition and the physiological ability of the suckling to digest it (Bauer et al., 2011). To date, most of the research focused on reduction of weaning problems *via* modification of the weaning diet. This resulted in different strategies being developed to modify the mode of feeding as well as the composition of feedstuff in terms of dietary components and biologically active ingredients (Heo et al., 2013). In the present study we used, however, a quite

different approach of inducing maturation changes in the digestive system, which are characteristic for weaning before the actual weaning occurred, employing lectin (phytohaemagglutinin, PHA) extracted from red kidney bean, *Phaseolus vulgaris* (Rådberg et al., 2001; Thomsson et al., 2007). Kidney bean lectin, a carbohydrate-binding protein, is constituted by a mixture of isoforms, PHA-$E_4$, PHA-$E_3L$, PHA-$E_2L_2$, PHA-$EL_3$, PHA-$L_4$, originally characterized by erythro- and leuco-agglutinating activity (Nordman et al., 1964; Leavitt et al., 1977). After gastric gavage it manifested strong promitotic activity in gut mucosa, and stimulated immune and endocrine systems in suckling piglets – described jointly as precocious maturation (Rådberg et al., 2001; Thomsson et al., 2007; Prykhod'ko et al., 2009). Importantly, maturation effects in the gastrointestinal tract were induced without systemic absorption of lectin (Linderoth et al., 2006).

In more recent studies it was shown that single administration of a lectin preparation led to a reduction of postweaning diarrhoea in piglets (Kiciak et al., 2010) and improved the animals' performance (Pieszka et al., unpublished data). However, the safety of kidney bean lectin preparations has not been studied so far, although earlier publications reported toxic effects of raw kidney bean consumption indisputably ascribed to lectin overdose (Noah et al., 1980; Rodhouse et al., 1990). Therefore, the aim of the present study was to evaluate the safety issues of kidney bean lectin preparation administered to suckling piglets under farm conditions.

## Material and methods

### Animals and management

All procedures were approved by the Local Ethics Committee for Animal Experimentation in Krakow (Poland).

The study was carried out at the Experimental Station (Unique Study Code: 02/2011) of the National Research Institute of Animal Production (Balice, Poland), which has a closed herd of about 50 sows. The experiment was conducted according to EFSA (2009) guidance on tolerance and efficacy studies, and the relevant parts of Commission Regulation (EC) No. 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No. 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorization of feed additives.

A total of four lactating sows of Polish Landrace × Polish Large White breed with suckling piglets (n = 30) were used. The sows were housed in single pens. The farrowing house and rooms used were typical for farrowing sows, with flat decks and slatted floors. The rooms were lit by a combination of day and artificial light. Each room was automatically air-conditioned/ventilated keeping the temperature between 22 and 27°C.

After weaning the piglets were housed in 4 pens with completely slatted floors in a climate-controlled weaning room. Mash diets were available to sows *ad libitum*. Mash creep diet was available to piglets *ad libitum* from postnatal day 7 (PD7), and starter diet from PD28. Small for gestational age neonates were excluded from the study but kept alive with the littermates. Piglets were randomized into three experimental groups: T1 – the control group, received 2 ml tap water by gavage on PD11 (n = 10), T2 – received 2 ml kidney bean extract (640 haemagglutination units (HAU)/piglet) water suspension by gavage on PD11 (n = 10), T3 – received 10 ml kidney bean extract (6400 HAU/piglet) water suspension by gavage on PD11 (n = 10).

Piglets were euthanized on PD42. Before euthanasia blood samples were withdrawn for biochemistry and haematology analysis from 6 (3 male and 3 female) piglets from each experimental group. Kidney bean lectin extract (50 g, Suilectin™, Biolek Sp. z o.o., Ożarów Mazowiecki, Poland) was mixed with 170 ml of water to obtain 320 HAU in 1 ml suspension, and a total of 2 ml of suspension (640 HAU/piglet, maximum recommended dose) was given to 11-day-old suckling piglets according to the producer's instructions (group T2). For the 10-fold dose of the maximum recommended dose of Suilectin™, a more concentrated suspension was prepared due to the small volume of the piglet stomach (group T3).

Sows were fed *ad libitum* with a regular diet and the piglets also had *ad libitum* access to creep feed from PD7 and were weaned on PD28. Sow and piglet diets were formulated without any antibiotics or other growth promoting alternatives such as essential oils, organic acids/salts, high Cu/Zn inorganic salts, etc. All nutrients were supplied at normal concentrations, for example not exceeding EU maximum permitted concentrations for trace minerals or vitamins. The diets (lactation, creep and prestarter feed) were calculated to be iso-nutritive, and to meet or exceed the nutrient requirements recommended for farrowing sows and suckling/weaning piglets (LfL, 2011). The ingredients, added

**Table 1.** The composition and nutritive value of compound feed for lactating sows

| Indices | Lactation feed, % |
|---|---|
| Ingredients | |
| wheat | 30.00 |
| wheat bran | 14.80 |
| maize | 35.00 |
| soyabean meal | 16.00 |
| global max premix[1] (4%) | 4.00 |
| lupro-mix (acidifiers) | 0.20 |
| Calculated analysis, % | |
| dry matter | 87.9 |
| crude protein | 15.80 |
| fat | 3.03 |
| fibre | 3.09 |
| Ca | 0.94 |
| Mg | 0.24 |
| P | 0.64 |
| available P | 0.16 |
| Na | 0.22 |
| K | 0.67 |
| ash | 2.29 |
| lysine | 0.907 |
| methionine | 0.298 |
| methionine + cysteine | 0.589 |
| threonine | 0.61 |
| tryptophane | 0.176 |
| isoleucine | 0.57 |
| leucine | 1.23 |
| valine | 0.69 |
| arginine | 0.91 |
| starch | 42.1 |
| ME, MJ· $kg^{-1}$ | 11.8 |

[1] composition of mineral-vitamin premix (Global Max premix, 4%, LNB Premix Production Plant – Cargill Poland Sp. z o.o., Kiszkowo, Poland) per kg: g: Na 50, Ca 208, P 42, Mg 10, lysine 60, methionine 12, valine 10, treonine 21; IU: vit. A 380000, vit. $D_3$ 50000; mg: vit. E 3500, vit. $K_3$ 125, vit. $B_1$ 57, vit. $B_2$ 152, vit. $B_3$ 1000, vit. $B_6$ 114, vit. $B_{12}$ 1.2, niacin 1000, folic acid 125, biotin 7.5, Fe 3300, Mg 1330, I 50, Zn 3010, Cu 510, Co 40, Se 12

**Table 2.** Feed composition and nutritive value of piglets mash creep and starter feed

| Indices | Mash creep feed, % | Starter feed, % |
|---|---|---|
| Ingredients | | |
| barley | 12.0 | 53.88 |
| wheat | 44.07 | 18 |
| soyabean meal | 24.0 | 20 |
| canola oil | 3.0 | 1 |
| skim milk powder | 9.0 | 4 |
| dried whey | 5.0 | 2 |
| mineral and vitamin mixture[1] | 0.5 | 0.5 |
| calcium phosphate | 1.1 | 1.0 |
| limestone | 0.8 | 1.1 |
| salt | 0.14 | 0.26 |
| L-lysine | 0.27 | 0.24 |
| DL-methionine | 0.12 | 0.02 |
| Nutrients per kg diet, % | | |
| dry matter | 88.4 | 88.4 |
| crude protein | 20.5 | 18.0 |
| crude fibre | 4.25 | 4.35 |
| crude fat | 3.37 | 2.83 |
| ash | 5.24 | 3.08 |
| Ca | 0.95 | 0.91 |
| Mg | 0.15 | 0.14 |
| P | 0.72 | 0.64 |
| available P | 0.47 | 0.41 |
| Na | 0.15 | 0.15 |
| K | 0.92 | 0.79 |
| lysine | 1.38 | 1.15 |
| methionine | 0.48 | 0.32 |
| methionine + cysteine | 0.78 | 0.61 |
| threonine | 0.76 | 0.66 |
| tryptophane | 0.25 | 0.21 |
| isoleucine | 0.84 | 0.71 |
| leucine | 1.57 | 1.32 |
| valine | 0.98 | 0.85 |
| arginine | 1.20 | 1.06 |
| starch | 33.6 | 39.1 |
| ME, MJ | 13.8 | 12.77 |

[1] Premix Forte (LNB Premix Production Plant – Cargill Poland Sp. z o.o., Kiszkowo, Poland) (0.5%) was added to provide the following nutrients per kg diet: IU: vit. A 15000, vit. $D_3$ 2000, vit. E 50; mg: vit. $B_1$ 1, vit. $B_2$ 5, vit. $B_{12}$ 30, biotin 60, folic acid 0.5, vit. K 2.5, pantothenic acid 15

vitamin/mineral premixtures, calculated and actual analyses of the diets are presented in Tables 1 and 2. The animals had free access to water from nipple drinkers (one per pen). Sows were kept in neighbouring standard pens equipped with an installation for sow and piglet protection, feeding trough, tap water nipple and box for piglets with infrared lamp. Individual body weights of piglets were recorded on PD0, PD7, PD14, PD28 and PD42. Pen weight gain, feed intake and feed conversion ratio were measured on PD7, PD14, PD28 and PD42. Health and mortality were monitored daily.

## Analytical methods

**Feeds.** All diets were prepared without inclusion of veterinary antibiotics and were analysed by the Central Laboratory of the National Institute of Animal Production Balice (Poland) using AOAC (2000) methods. The composition and nutritive values of sow and piglet diets are given in Tables 1 and 2, respectively.

**Blood sample analysis.** On PD42 (i.e. 32 days after administration of water or kidney bean extract) immediately before euthanasia, blood (10 ml) was withdrawn from the right external vein into plastic tubes containing EDTA $K_3E$ (2 ml, EQUIMED, Kraków, Poland) for haematology analysis, and into lithium heparin-pretreated tubes (4 ml, EQUIMED, Kraków, Poland) for plasma biochemistry and for serum cortisol RIA. Blood was chilled to +4°C, and a portion for biochemistry analyses was centrifuged for 10 min (4000 rpm, +4°C). Plasma aliquots were

stored for further analyses (–20°C). Haematology analyses were performed on the day of blood sampling. Serum samples for cortisol RIA were kept frozen (–20°C) until analysis.

The following haematology analyses were performed in EDTA-conserved blood: haemoglobin concentration (HGB, mmol · $l^{-1}$), haematocrit (HCT, %), red blood cell count (RBC, $10^{12}$ · $l^{-1}$), white blood cell count (WBC, $10^{9}$ · $l^{-1}$), mean red blood cell volume (MCV, fl), mean red blood cell haemoglobin mass (MCH, pg), mean red blood cell haemoglobin concentration (MCHC, mmol · $l^{-1}$), red blood cell distribution width - anisocytosis index (RDW, %), platelet count (PLT, $10^{9}$ · $l^{-1}$), and qualitative analysis of white and red blood cells.

Morphological analysis of blood was performed using a haematological analyser ADVIA 2120 (Siemens, Erlangen, Germany). Biochemical analysis of blood plasma was performed using a spectrophotometer Beckman DU-640 (Fullerton, CA, USA). Plasma was obtained by centrifugation of whole blood. Concentrations of glucose, total protein and urea were determined in plasma together with the activity of alanine transferase (ALAT) and aspartate aminotransferase (ASAT) (PZ Cormay S.A., Łomianki, Poland). The concentration of cortisol in serum was determined by RIA using a Beckman apparatus and cortisol RIA kit (Immunotech, Marseille, France).

**Post mortem examination and histopathology.** The piglets were sacrificed by pentobarbiturate overdose (*Thiopentalumnatricum,* Thiopental 1 g, Sandoz, Switzerland), and sectioned. During necropsy, the visceral organs, in particular stomach, small and large intestine, liver, pancreas, kidneys, heart and lungs, were inspected visually by the veterinarian, measured for size and weight, and tissue samples were taken on 4% buffered formaldehyde solution for histology. Tissue samples were fixed in 4% buffered formaldehyde for 48 h. In the next step, all samples were washed in 70% ethyl alcohol and stored for further analyses. Using an automatic tissue processor (STP 120, Mikrom, Walldorf, Germany), the samples were then dehydrated in a increasing 70% to 99.8% ethyl alcohol series, twice overexposed in xylene and twice saturated in paraffin. Immediately after the end of the last incubation in paraffin, all samples were embedded in liquid paraffin to form paraffin blocks. Paraffin blocks were cut into slides of 5 µm thickness on a rotation microtome (HM 325, Carl Zeiss AG, Jena, Germany), and stained with haematoxylin and eosin for light microscopy analysis (BX61, Olympus, Tokyo, Japan). Routine histological analysis of tissue slides was performed by an anatomo-pathologist.

**Gut content sampling and bacteriology studies.** During necropsy, a 10 cm intestinal loop consisting of a mid-segment of the ileum was closed between two cotton threads and dissected. The closed loops were immediately transported on ice to the microbiology laboratory for further routine analyses (total bacteria, *Escherichia coli*, *Enterococcus* spp., *Lactobacillus* spp., *Clostridium* spp. counts, colony forming unit (CFU) · $g^{-1}$)). The ileal content was homogenized in sterile PBS and then serial 10-fold dilutions were made, also using sterile PBS. Aliquots (0.1 ml) of the appropriate dilutions were plated on selective agars. All samples were examined for total aerobic counts on Columbia agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etiole, France), for *Lactobacillus* spp. on Rogosa agar (Oxoid - Thermo Fisher-Scientific, Waltham, MA, USA), for *E. coli* on MacConkey agar (bioMérieux, Marcy l'Etiole, France), for *Clostridium* spp. on TSC agar supplemented with D-cykloserine 400 µg · $ml^{-1}$ (Merck KGaA, Darmstadt, Germany), for *Enterococcus* spp. on kanamycin esculin azide agar (Merck, KGaA, Darmstadt, Germany). Total aerobes, lactobacilli, enterococci and *Clostridium* spp. were incubated for 72 h, and *E. coli* for 24 h. Anaerobic atmospheric conditions generated by a BBL™ GasPak system (Becton Dickinson and Company, Franklin Lakes, NJ, USA) were used for lactobacilli and *Clostridium* spp., while the remaining bacteria were cultured under aerobic conditions. All incubations were carried out at 37°C. After the incubation, colonies were enumerated and the CFU · $g^{-1}$ of ileal content was calculated.

### Statistical analysis

One-way-analysis of variance ANOVA followed by Tukey's test was used for statistical analysis of pig performance, and blood biochemical and haematology parameters. $P \leq 0.05$ was statistically significant, while $0.05 < P \leq 0.10$ was a near-significant trend. Data were analysed using the Statgraphics® Plus ver. 5.1 statistical package (Statgraphics, 2001).

## Results

Veterinarians supervising the study did not note any clinical manifestations in piglets during the entire study period. Clinical observations made on PD42 did not show any gastrointestinal or respiratory tract disturbances. The animals were clinically healthy. No piglets died during the experiment. No significant effect of administration of kidney bean extract, recommended and 10x recommended dose, on the body weight of piglets during the rearing period to PD42 was observed (Table 3). The study design did not

**Table 3.** Effect of water (T1) and two doses of kidney bean extract (T2 and T3) on piglet's performance

| Parameter | T1 control | T2 640 HAU/pig | T3 6400 HAU/pig | SEM | |
|---|---|---|---|---|---|
| Number of piglets | 10 | 10 | 10 | – | – |
| Body weight at day | | | | | |
| 1 (birth) | 1.35 | 1.22 | 1.48 | 0.05 | 0.15 |
| 11 | 3.93 | 3.03 | 3.16 | 0.1 | 0.06 |
| 14 | 4.73 | 3.79 | 3.83 | 0.21 | 0.14 |
| 28 (weaning) | 8.78 | 7.23 | 7.21 | 0.38 | 0.17 |
| 42 | 9.83 | 8.13 | 8.18 | 0.39 | 0.13 |
| Average daily gain, g | | | | | |
| 1–11 | $234^{b}$ | $164^{a}$ | $153^{a}$ | 12.81 | 0.01 |
| 1–14 | 231 | 252 | 224 | 19.72 | 0.83 |
| 14–28 | 289 | 246 | 240 | 14.69 | 0.34 |
| 28–42 | 75 | 63 | 69 | 5.85 | 0.75 |
| Average feed intake, g/d/piglet | | | | | |
| 11–28 | 47 | 36 | 49 | – | – |
| 28–42 | 164 | 161 | 164 | – | – |
| FCR per 1 kg of body weight gain. kg | | | | | |
| 11–28 | 0.169 | 0.148 | 0.205 | – | – |
| 28–42 | 2.190 | 2.517 | 2.371 | – | – |

No. replicates = 30 (10 replicates (piglets)/treatment); SEM – standard error of mean, FCR – feed conversion rate, $^{AB, ab}$ means with different superscipts within a row are significantly different at $P \leq 0.01$ or $P \leq 0.05$, respectively

**Table 4.** Effect of water (T1) and two doses of kidney bean extract (T2 and T3) on haematology parameters

| Parameter | T1 control | T2 640 HAU/pig | T3 6400 HAU/pig | *P* |
|---|---|---|---|---|
| WBCP, $x10^3$ cells · $\mu l^{-1}$ | 26.27 ± 4.90 | 23.19 ± 2.47 | 25.69 ± 2.26 | 0.28 |
| WBCB, $x10^3$ cells · $\mu l^{-1}$ | 25.32 ± 4.12 | 22.89 ± 2.84 | 24.14 ± 2.60 | 0.45 |
| RBC, $x10^6$ cells · $\mu l^{-1}$ | 7.87 ± 0.40 | 7.20 ± 1.07 | 7.41 ± 0.88 | 0.38 |
| HGB, g · $dl^{-1}$ | 13.75 ± 1.20 | 13.73 ± 1.47 | 13.32 ± 1.42 | 0.82 |
| HCT, % | 44.82 ± 3.13 | 43.95 ± 4.03 | 42.94 ± 3.63 | 0.67 |
| MCV, fL | 56.97 ± 3.54 | 59.57 ± 2.31 | 57.38 ± 4.00 | 0.37 |
| MCH, pg | 17.45 ± 1.51 | 18.82 ± 0.56 | 17.77 ± 1.73 | 0.22 |
| MCHC, g · $dl^{-1}$ | 30.63 ± 0.83 | 31.55 ± 0.48 | 30.93 ± 1.31 | 0.25 |
| CHCM, g · $dl^{-1}$ | 30.98 ± 1.22 | 31.83 ± 0.46 | 31.17 ± 1.28 | 0.36 |
| RDW, % | 16.53 ± 1.99 | 14.90 ± 0.96 | 15.43 ± 0.98 | 0.15 |
| PLT, $x10^3$ cells · $\mu l^{-1}$ | 426.00 ± 124.62 | 434.67 ± 94.37 | 504.67 ± 74.48 | 0.35 |
| MPV, fL | 9.36 ± 0.68 | 8.25 ± 0.75 | 8.22 ± 1.49 | 0.051 |
| NEUT, % | 44.83 ± 6.17 | 38.68 ± 5.26 | 40.00 ± 7.74 | 0.25 |
| LYM, % | 47.77 ± 5.63 | 56.25 ± 5.62 | 52.70 ± 7.10 | 0.08 |
| MONO, % | $4.23 \pm 1.05^{bB}$ | $2.50 \pm 0.46^{aA}$ | $4.12 \pm 0.79^{bB}$ | 0.003 |
| EOS, % | $1.60 \pm 0.72^{bB}$ | $0.98 \pm 0.17^{abAB}$ | $0.65 \pm 0.21^{aA}$ | 0.006 |
| LUC, % | 0.98 ± 0.69 | 0.63 ± 0.34 | 1.03 ± 0.49 | 0.38 |
| BASO, % | 0.63 ± 0.12 | 0.72 ± 0.33 | 0.58 ± 0.23 | 0.64 |

WBC – white blood cells, RBC – red blood cells, HGB – haemoglobin, HCT – haematocrit, MCV – mean red cell volume, MCH – mean red cell haemoglobin mass, MCHC – mean red cell haemoglobin concentration, CHCM – haemoglobin concentration mean, RDW – red cell distribution width, PLT – platelets, MPV – platelet volume, NEUT- neutrophils, LYM – lymphocytes, EOS – eosinophil, LUC – large unstained cells, BASO – basophil; mean ± SD (n = 6); $^{AB, ab}$ see Table 3

allow employing statistical tests to analyse feed intake and feed conversion ratio, although numerical values are given in Table 3.

Haematology and blood biochemistry data are given in Tables 4 and 5. All results were in the physiological range for piglets, although some statistically significant differences between groups in monocyte (physiological range: 0-10%) and eosinophil (physiological range: 0–1.5%) counts (Table 4) as well as in blood glucose (physiological range: 66–116 mg · $dl^{-1}$) and ALAT (physiological range: 22–45 U · $l^{-1}$) (Table 5) were found (Latimer et al., 2003; The Merck Veterinary Manual online – reference value section). Piglets from group T2 had the highest blood cortisol concentration (Table 5), although that measured in group T3 did not differ from than in group T1 (control).

No major pathological changes were observed during necropsy or in light microscopy of histology slides (Tables 6 and 7). Hyperaemia of internal organs was observed in some animals in all groups.

**Table 5.** Effect of water (T1) and two doses of kidney bean extract application (T2 and T3) on blood serum biochemical parameters

| Parameter | T1<br>control | T2<br>640 HAU/pig | T3<br>6400 HAU/pig | *P* |
|---|---|---|---|---|
| Glucose, mg · dl$^{-1}$ | 79.52 ± 7.87$^{a}$ | 91.97 ± 4.28$^{b}$ | 86.52 ± 9.93$^{ab}$ | 0.04 |
| Urea, mg · dl$^{-1}$ | 36.86 ± 3.24 | 31.36 ± 4.58 | 35.04 ± 6.33 | 0.17 |
| Total protein, g · dl$^{-1}$ | 6.43 ± 0.73 | 5.93 ± 0.65 | 6.53 ± 0.89 | 0.36 |
| ALAT, U · l$^{-1}$ | 25.10 ± 4.24$^{bB}$ | 14.17 ± 3.53$^{aA}$ | 19.69 ± 4.56$^{abAB}$ | 0.001 |
| ASAT, U · l$^{-1}$ | 51.33 ± 16.26 | 49.63 ± 12.97 | 51.46 ± 10.66 | 0.96 |
| Cortisol , ng · ml$^{-1}$ | 21.16 ± 4.44$^{a}$ | 33.86 ± 8.86$^{b}$ | 25.61 ± 8.93$^{ab}$ | 0.02 |

$^{AB,ab}$ means with different superscipts within a row are significantly different at $P \leq 0.01$ or $P \leq 0.05$, respectively; mean ± SD

**Table 6.** Effect of water (T1) and two doses of kidney bean extract (T2 and T3) on post mortem results in the heart, abdominal cavity, spleen, pancreas, and small intestine

| Organs | T1<br>control | T2<br>640 HAU/pig | T3<br>6400 HAU/pig |
|---|---|---|---|
| Lungs | Hyperaemia (4/6)<br>Focal inflammation (1/6)<br>No changes 1/6 | No changes (4/6)<br>Hyperaemia (2/6) | No changes (2/6)<br>Focal inflammation (1/6)<br>Pulmonary oedema 1/6<br>Petechiae in lungs 2/6 |
| Kidneys | Hyperaemia (1/6)<br>No changes (5/6) | No changes (4/6)<br>Congestion of renal pelvis (2/6) | No changes (6/6) |
| Liver | Hyperaemia (3/6)<br>Congested (1/6)<br>No changes (2/6) | No changes (5/6)<br>Liver fragility (1/6) | No changes (4/6)<br>Liver fragility (2/6) |
| Stomach | No changes (2/6)<br>Damage mucosa (2/6)<br>Necrotic changes (2/6) | No changes (3/6)<br>Damage mucosa (2/6)<br>Gastric ulcers (1/6) | No changes (3/6)<br>Inflammatory changes (2/6)<br>Ulcers of the stomach (1/6) |
| Small intestine | No changes (5/6)<br>Congested intestine (1/6) | No changes (4/6)<br>Inflammation (2/6) | No changes (6/6) |

Table 7. Effect of water (T1) and two doses of kidney bean extract (T2 and T3) on histopathology results

| Organs | T1<br>control | T2<br>640 HAU/pig | T3<br>6400 HAU/pig |
|---|---|---|---|
| Stomach | No changes (1/6)<br>Mild superficial infiltration with mononuclear cells (5/6) | Mild superficial infiltration with mononuclear cells (6/6)<br>Hyperplasia of gastric glands (1/6) | Mild superficial infiltration with mononuclear cells (6/6) |
| Small intestine | No changes (1/6)<br>Villi flattening (5/6) | No changes (2/6)<br>Villi flattening (4/6)<br>Infiltration with eosinophils (2/6) | No changes (1/6)<br>Villi flattening (5/6) |
| Pancreas | No changes (1/6)<br>Zymogen content increase (5/6) | No changes (1/6)<br>Zymogen content decrease (5/6)<br>Focal necrosis of acinar cells (1/6) | Zymogen content decrease (6/6)<br>Focal necrosis of acinar cells (1/6) |
| Liver | Mild cloudy swelling (6/6) | Mild cloudy swelling (6/6) | No changes (1/6)<br>Mild cloudy swelling (5/6) |
| Heart | No changes (5/6)<br>Focal degeneration of cardiomyocytes (1/6) | No changes (5/6)<br>Focal degeneration of cardiomyocytes (1/6) | No changes (5/6)<br>Perivascular infiltration with lymphocytes (1/6) |
| Spleen | No changes (3/6)<br>Thickening of arteries wall (3/6) | No changes (3/6)<br>Thickening of arteries wall (3/6) | No changes (2/6)<br>Thickening of arteries wall (4/6) |
| Lung | Focal atelectasis (5/6)<br>Perivascular infiltration with lymphocytes (4/6) | Focal atelectasis (6/6)<br>Peribronchial infiltration with lymphocytes (2/6) | No changes (3/6)<br>Focal atelectasis (2/6)<br>Perivascular infiltration with lymphocytes (1/6) |
| Kidney | Mild cloudy swelling (6/6)<br>Hyperplasia of arterial wall (1/6) | Mild cloudy swelling (6/6) | No changes (1/6)<br>Mild cloudy swelling (5/6) |

**Table 8.** Effect of water (T1) and two doses of kidney bean extract application (T2 and T3) on bacterial counts (CFU · $g^{-1}$) in ileal content

| Item | T1 control | T2 640 HAU/pig | T3 6400 HAU/pig |
|---|---|---|---|
| Total aerobic bacteria count | 2.2–3.13 × $10^8$ | 1.7–7.38 × $10^8$ | 1.04–7.1 × $10^8$ |
| *Escherichia coli,* | 3.8–6.4 × $10^7$ | 1.6–5.1 × $10^7$ | 1.1–4.5 × $10^7$ |
| *Enterococcus* spp. | 2.2–5.1 × $10^8$ | 1.04–3.75 × $10^8$ | 1.26–6.4 ×$10^8$ |
| *Lactobacillus* spp. | 2.82–6.88 × $10^8$ | 1.36–6.7 ×$10^8$ | 1.34– 6.7 ×$10^8$ |
| *Clostridium* spp. | 2.5–6.0 × $10^3$ | 3.8–8.2 × $10^4$ | 1.1 – 6.5 × $10^3$ |

CFU – colony-forming unit

Bacteriology analyses revealed no significant differences between the three examined groups of piglets in regard to aerobic and anaerobic bacteria counts (Table 8).

## Discussion

In contrast to previously published data on acute toxicity (acute 3-day diarrhoea) following consumption of raw kidney beans (Noah et al., 1980; Rodhouse et al., 1990), we have shown that a single dose of kidney bean lectin extract applied to induce precocious maturation of the gastrointestinal tract in neonatal piglets is a safe procedure. In the present study no pigs died or had diarrhoea following kidney bean lectin administration. Several changes were observed during the study, among others in haematology and blood biochemistry, as compared with controls, however, it should be emphasized that all results, including the results obtained in the group treated with the high dose of kidney bean lectin, were within the physiological range for piglets. Nevertheless, it is known that lectins from legumes, including kidney bean lectins, may provoke immune system development (Prykhod'ko et al., 2009). Plant lectins may increase allergic reactions in humans and animals (Cordain et al., 2000; Lavelle et al., 2000). The discrepancies between the literature and our data may be explained by the fact that kidney bean lectin was given just once to our piglets, whereas in other studies it was applied repeatedly. Also, we did not study the kinetics of the process - just one time point has to be assessed according to EFSA guidelines (Commission Regulation (EC) No. 429/2008).

Other works suggest the antihiperglycaemic activity of red kidney bean extracts and lectin PHA (Baintner et al., 2003). One of the hypotheses says that the mechanism of this activity relies on the stimulation of pancreatic secretion of α-amylase in rats (Baintner et al., 2003); this should result in the accelerated metabolism of ingested starch and, in turn, an increase in glycaemia. The higher levels of glucose in the blood of piglets receiving kidney bean lectin extract in our study may be a sign of high reactivity of the exocrine pancreas in these animals as compared with the control group. These binding results in the stimulation of the release of cholecystokinin and glucagon-like peptides (Herzig et al., 1997; Rådberg et al., 2001), two hormones known to stimulate exo- and endocrine pancreas function. According to Grant et al. (1987), a high lectin content induces glycogen catabolism and as a result of this process, serum glucose levels increase. In addition, we observed a statistically significant increase in the level of cortisol in piglets treated with the lower dose of kidney bean lectin extract in comparison with the control group. Presumably, the lower dose of extract enhanced the ability of the pig to respond to stress by increasing circulating levels of cortisol. A high cortisol concentration suppresses immune system function (Kelley, 1988), resulting in a lower level of monocytes. Additionally, the high cortisol concentration in group T2 may also contribute to increasing the glucose level by enhancing gluconeogenesis and inhibition of extrahepatic glucose utilization (Mcmahon et al., 1988). However, the effect was not dose-dependent, since a 10-fold higher dose of extract did not produce any elevation in plasma cortisol. It seems that the changes in the cortisol profile were induced by yet undefined factors other than application of kidney bean lectin extract.

The most striking phenomenon is a significant reduction in ALAT activity suggesting induction of a liver-protecting mechanism(s) following lectin treatment. Interestingly, a tendency towards a decrease was also observed with the high dose of kidney bean lectin extract. Presumably, it could be the effect of enhanced mucosal integrity due to induction of precocious maturation of the gut (Rådberg et al., 2001).

In a parallel efficacy study on piglets involving a single application of the recommended dose of kidney bean extract, we observed significantly higher average feed intake, improved feed utilization and higher weight gain (Pieszka et al., unpublished data). Similar effects were reported earlier (Valverde-Piedra et al., 2006; Kiciak et al., 2010). In the present experimental protocol, however, it was difficult to expect so positive results due to the low number of treatments and animals per treatment. In the present study there were no significant differences in body weight, feed intake or feed conversion ratio among the T1, T2 and T3 groups, which contrasts with the

performance of the animals in the aforementioned efficacy studies. Nevertheless, our results clearly show that administration of a dose 10-fold higher than the recommended dose (group T3) did not produce negative effects in terms of either mortality/ morbidity or body weight and feed intake during one month after application of the kidney bean lectin extract, which was the main aim of the present study. Accordingly, no major pathological anatomical, histological or bacteriological changes were observed when applying the large and recommended doses of kidney bean extract.

## Conclusions

A one-month-long tolerance study using the recommended dose (640 haemagglutination (HAU)/ pig) and 10-fold recommended dose (6400 HAU/ pig) of kidney bean extract demonstrated no toxic effects on animal health, haematological and blood plasma biochemistry parameters, post mortem studies, as well as on ileal aerobic and anaerobic bacteria counts in piglets.

## Acknowledgements

The authors are grateful to Maria Siemińska and Czesława Janczy for skilful technical support. The study was supported by Biolek Sp. z o.o., Ożarów Mazowiecki (Poland).

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