The effects of adding lucerne protein concentrate to diets on the reproductive traits and blood metabolic profiles of sows and piglets

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ABSTRACT. The aim of the study was to estimate the effects of supplementing different amounts of lucerne protein concentrate (LPC) on performance, blood haematological and biochemical parameters of sows and their offspring. LPC was added in amounts of 1.5% or 3.0% to the feed mixtures for sows during gestation and lactation (Trial I, continuous use of LPC) or only during gestation or lactation (Trial II, periodic use of LPC) and for their offspring from birth to day 84. Production results were based on: number of liveborn and weaned piglets, daily gains, average feed intake, feed conversion ratio and mortality. Blood samples were collected to estimate haematological and biochemical indices. Compared with the control groups, addition of 3.0% LPC during gestation significantly increased ($P \leq 0.05$) average piglet birth weight, number of liveborn piglets and weaned piglets at day 28. Diets containing 1.5% and 3.0% LPC decreased losses of piglets by day 84 ($P \leq 0.05$). The diet including LPC significantly ($P \leq 0.05$) increased haematological indices: red blood cell count, haematocrit level, haemoglobin content, and biochemical parameters, especially alkaline phosphatase activity ($P \leq 0.01$) in sow and piglet serum. The noticeable effect of LPC on haematological indices could be useful, especially in the piglet rearing period. Our results show that the best effects could be obtained by adding 3.0% LPC to the sows' diets, but 1.5% of LPC can also be recommended in pig nutrition.

Introduction

Removal of antibiotic growth promoters (AGPs) from the market and banning their use in feeds has promoted studies on alternative sources of biologically active supplements that can be effectively incorporated into pig diets (Lainea et al., 2014). Intensive research has shown that various lucerne-based preparations should be considered as feed additives (Bourdon et al., 1980; Ueda and Ohshima, 1989; Grela et al., 2008; Davys et al., 2011).

Lucerne protein concentrate (LPC) contains about 55% crude protein, over 1200 mg of xanthophylls per kilogram of preparation and a high number of active substances, including saponin glycosides (2%–3%), polyphenolic compounds with oestrogenic activity (biochanin A, daidzein), vitamins (A, B_1, B_6, B_{12}, C, E, K), provitamins (β-carotene) and minerals (EFSA, 2009; Grela and Pietrzak, 2014). Such a potent source of biologically active substances in LPC can affect pig metabolism and thus, performance and blood metabolic profile.

The objective of the present research was to determine the effect of supplementing lucerne protein concentrate (LPC) to sow feeds during gestation and lactation, as well as in the piglet rearing period (to 84 days of age) on performance effects and blood haematological and biochemical parameters. The studies examined two LPC levels (1.5% and 3.0%) throughout the whole reproductive and rearing period or a 3.0% periodic level only during gestation or lactation.

Material and methods

Animals, dietary treatment and management

Two dietary treatments were performed on 60 crossbreed sows (Polish Landrace × Polish Large White) mated to Duroc boars and their offspring to the end of the piglet rearing period (84 days of age) during March–October 2011 (Trial I) and January–August 2012 (Trial II). In Trial I 60 sows in the second reproductive cycle were randomly allocated to one of three feeding groups: control (without additive), 1.5% LPC (E-15), 3.0% LPC (E-30) for the entire experimental period. Trial II included the same 60 sows in their third reproductive cycle and three feeding groups, i.e. control, group E-30G that received a mixture with 3.0% LPC additive during gestation, and group E-30L fed the 3.0% LPC-supplemented mixture only during lactation. From the beginning of creep feeding to day 14 post weaning, the piglets ate prestarter feeds, while from days 43 to 84 of age, the starter diet. In each dietary treatment the piglets during creep feeding and after weaning were allocated to 3 groups: control (no supplement), piglets from E-15 and E-30 were fed a mixture with 1.5% or 3.0% LPC additive, respectively, whereas the animals from E-30G and E-30L received a 3.0% LPC-supplemented diet. The chemical composition and concentration of active ingredients of LPC are shown in Table 1, formulation and nutritive value of diets for sows are presented in Table 2 and in Table 3 for piglets. The changes in the body weight of sows were recorded by weighing animals at mating, day 110 of gestation, 2 days post partum, and after weaning the piglets, whereas feed intake was measured during gestation and lactation. The number of piglets born and reared, their weight at birth, on weaning and on day 84 of age at the end of the rearing period were recorded. In addition, feed intake was measured during creep feeding and after weaning to day 84 of life, and the causes of piglet mortality were determined.

Table 1. Chemical composition, mineral concentration and active ingredients content in lucerne protein concentrate (LPC) (Grela and Pietrzak, 2014)

Nutrients, g · kg⁻¹		Macroelements, g · kg⁻¹	
Dry matter	910.4	Calcium	32.9
Crude ash	102.3	Phosphorus	7.9
Ether extract	103.7	Magnesium	1.5
Crude fibre	5.9	Potassium	7.4
Crude protein	533.9	Sodium	0.13
N-free extractives	164.6		
Vitamins and saponins, mg · kg⁻¹		Microelements, mg · kg⁻¹	
α-tocopherol	428.2	Iron	497.0
β-carotene	303.7	Manganese	81.8
Vitamin K	95.3	Copper	10.2
L-canavanine	3.2	Zinc	19.4
Saponins, g · kg⁻¹	10.39		

Feed and blood analysis

Feed samples were assayed for the contents of basic nutrients and detergent fibre fraction (AOAC, 2012). The energy value of diets was calculated using the equation proposed by Kirchgessner and Roth (1983). Blood for laboratory evaluation was collected from the external jugular vein (*vena jugularis externa*) from eight sows on day 84 of gestation and 21 of lactation, eight piglets (28 days of age) and weaner (84 days of age) from each group. The following haematological parameters were determined in whole blood using an Abacus Junior Vet analyzer: red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Ht), white blood cell count (WBC) as well as their percentages: lymphocytes (LYM), middle-sized cells (MID) and granulocytes (GRA). Blood plasma was analysed for concentration of glucose (GLU), total protein (TP), blood urea nitrogen (BUN), uric acid (UA) and triacylglycerols (TG) using a colorimetric method and monotests from PZ Cormay SA (Łomianki, Poland). The urea level and activity of the following enzymes: lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were established by the kinetic method using monotests from PZ Cormay SA (Łomianki, Poland). The readings of the determined biochemical parameters in blood plasma were made using a CARY 50 Spectrophotometer (Varian, Inc., Palo Alto, CA, USA).

Statistical analysis

The hypothesis of the empirical normal distribution of recorded traits was verified using Shapiro-Wilk test. It was found no statistically significant deviations from the normal distribution. The data were

evaluated statistically using a general linear model of one-way analysis of variance. Duncan's test was applied for the multiple comparisons between treatment means. These computations were performed by the use of the Statistica Software v.6.1 (2003).

Results

Animal performance

Substitution of soyabean meal by LPC in the experimental diets for sows (Table 2) and piglets (Table 3) contributed only slightly to nutrient content variation. The initial body weight (BW) of sows in both dietary treatments averaged 170 kg (Table 4). In Trial I in late gestation (110 days), the body weight of animals from E-15 and E-30 differed significantly ($P \leq 0.05$) as compared with the control sows. The sows fed the 1.5% and 3% LPC-supplemented diets were heavier by 4.1 and 5.9 kg, respectively. In Trial II, on day 110 of gestation, a significantly higher weight of sows was found only in group E-30G. In both experiments, sow BW after farrow-

ing did not differ significantly. The sows that after lactation received 3.0% LPC in groups E-30 and E-30L showed a smaller weight loss compared with the control. Feed intake in both treatments during gestation and lactation did not differ significantly among the animal groups under study. The production performance of piglets is presented in Table 4. In Trial I, the sows receiving 3.0% LPC had a significantly higher number of liveborn piglets than in the control, and the average piglet birth weight was also significantly higher in both experimental groups: by 5.5% and 6.7% in groups E-15 and E-30, respectively. After 28 lactation days, a higher number of weanling piglets ($P \leq 0.05$) from the sows in the experimental groups (E-15 and E-30) was recorded as compared with the control. Piglet weaning weight did not differ significantly among the groups, however. It is noteworthy that during the rearing time with sows, a lower piglet mortality rate was noted in the treatment groups in which the sows were given the lucerne protein concentrate at 15 and 30 g per kilogram of mixture. Piglet losses were lower by

Table 2. Chemical composition and nutritive value of mixtures for sows in gestation and lactation period

Indices	Reproductive period					
	LP (gestation)			LL (lactation)		
	experimental groups					
	control	E-15	E-30	control	E-15	E-30
Ingredients, g · kg⁻¹						
barley	555	555	555	285	285	285
wheat	100	100	100	300	300	300
oat	200	200	200	100	100	100
dried grass	50	50	50	50	50	50
fish meal	–	–	–	30	30	30
soyabean meal	60	45	30	180	165	150
soyabean oil	–	–	–	20	20	20
lucerne concentrate PX	–	15	30	–	15	30
fodder chalk	11	11	11	12	12	12
2-calcium phosphate	10	10	10	10	10	10
NaCl	4	4	4	4.0	4.0	4.0
vitamin-mineral premix[1]	10	10	10	10	10	10
lysine HCl	–	–	–	3	3	3
Nutritional value (determined), g · kg⁻¹ DM						
crude protein	143.3	143.4	143.5	191.6	191.7	191.8
lysine	5.49	5.53	5.54	12.48	12.51	12.55
crude fibre	59.6	59.5	59.5	47.3	47.3	47.2
NDF	273.8	273.5	273.4	241.6	241.5	241.3
ADF	93.6	93.7	93.7	84.2	84.2	84.3
ADL	12.7	12.7	12.7	9.7	9.7	9.7
Ca	7.44	7.46	7.54	9.24	9.27	9.28
total P	5.56	5.60	5.63	6.40	6.41	6.44
metabolizable energy[2], MJ · kg⁻¹	11.81	11.81	11.81	12.42	12.42	12.42

E-15 – 15 g LPC per kg mixture; E-30 – 30 g LPC per kg mixture; [1] vitamin-mineral premix: g: Ca 5 (CaCO₃)/(Ca(H₂PO₄)/(CaI₂), P 1.3 (Ca(H₂PO₄); mg: Fe 100 (Fe(SO₄) x 7H₂O), Zn 100 (ZnO), Cu 23 (CuSO₄ x 5H₂O), I 1.2 (CaI₂), Se 0.3 (Na₂SeO₃); IU: vit. A 8.000; vit. D₃ 1.000; vit. E 60, vit. K 0.60; riboflavin 4, niacin 22, pantothenic acid 15, vit. B₁₂ 0.02, choline 750; NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin; [2] metabolizable energy according to the equation of Kirchgessner and Roth (1983)

Table 3. Chemical composition and nutritive value of mixtures for piglets

Indices	Prestarter			Starter		
	experimental groups					
	control	E-15[1]	E-30[1]	control	E-15	E-30
Ingredients, g · kg⁻¹						
barley	–	–	–	220.0	220.0	220.0
wheat	509.0	509.0	509.0	412.5	412.5	412.5
maize	200.0	200.0	200.0	200.0	200.0	200.0
skimmed milk powder	80.0	80.0	80.0	–	–	–
fish meal	50.0	50.0	50.0	30.0	30.0	30.0
soyabean meal	40.0	25.0	10.0	100.0	85.0	70.0
lucerne concentrate PX	–	15.0	30.0	–	15.0	30.0
soyabean oil	30.0	30.0	30.0	10.0	10.0	10.0
saccharose	50.0	50.0	50.0	–	–	–
fodder chalk	5.5	5.5	5.5	6.0	6.0	6.0
1-calcium phosphate	4.0	4.0	4.0	4.0	4.0	4.0
NaCl	2.8	2.8	2.8	3.0	3.0	3.0
vitamin-mineral premix[2]	20.0	20.0	20.0	10.0	10.0	10.0
lysine HCl	8.0	8.0	8.0	4.3	4.3	4.3
DL-methionine	0.7	0.7	0.7	0.2	0.2	0.2
Nutritional value (determined), g · kg⁻¹ DM						
crude protein	224.7	224.8	224.9	183.5	183.6	183.8
lysine	15.67	15.72	15.75	11.26	11.29	11.31
crude fibre	24.1	24.1	24.0	31.7	31.6	31.5
NDF[3]	118.4	118.5	118.5	176.1	176.3	176.2
ADF[3]	42.2	42.4	42.3	44.9	44.8	44.7
ADL[3]	5.42	5.41	5.42	16.7	16.7	16.7
Ca	6.42	6.44	6.59	5.13	5.17	5.16
total P	5.65	5.69	5.73	5.19	5.21	5.20
metabolizable energy[4], MJ · kg⁻¹	13.12	13.12	13.12	12.53	12.53	12.53

[1] see Table 2; [2] vitamin-mineral premix: g: Ca 5 $(CaCO_3)/(Ca(H_2PO_4)/(CaI_2)$, P 1.3 $(Ca(H_2PO_4)$, mg: Fe 100 $(Fe(SO_4)$ x $7H_2O)$, Zn 100 (ZnO), Cu 23 $(CuSO_4$ x $5H_2O)$, I 1.2 (CaI_2), Se 0.3 (Na_2SeO_3); IU: vit. A 8.000, vit. D_3 1.000, E 60, vit. K 0.60, riboflavin 4, niacin 22, pantothenic acid 15, vit. B_{12} 0.02, choline 750; [3] see Table 2; [4] metabolizable energy according to the equation of Kirchgessner and Roth (1983)

Table 4. Sows body weight and feed intake during gestation and lactation

Indices	Trial I			Trial II			SEM
	control	E-15[1]	E-30[1]	control	E-30G	E-30L	
Sows, No.	20	20	20	20	20	20	–
Body weight, kg							
at mating	169.4	170.2	168.9	172.8	173.4	172.5	5.63
at 110 d of gestation	194.3ᵃ	198.4ᵇ	200.2ᵇ	197.5ᵃ	202.8ᵇ	198.4ᵃ	5.96
after farrowing	178.1	179.7	178.4	181.4	183.9	182.8	6.14
at weaning	171.5ᵃ	173.1ᵃᵇ	175.4ᵇ	174.3ᵃ	177.5ᵃᵇ	179.5ᵇ	5.72
Feed intake, kg per sow							
during gestation	2.65	2.67	2.71	2.66	2.69	2.65	0.09
during lactation	5.88	5.91	5.95	5.84	5.91	5.83	0.28

[1] see Table 2; E-30G – 30 g LPC per kg gestation mixture; E-30L – 30 g LPC per kg lactation mixture; SEM – standard error mean; ᵃ,ᵇ values in experiment I or II in the same rows with different letters differ significantly ($P \leq 0.05$)

1.76% and 2.19%, respectively, against the control. During lactation, piglet weight gains did not differ significantly among the groups, whereas the total feed intake of piglets during lactation was significantly lower in group E-30 than in the other groups. The sows fed the 3.0% LPC-supplemented diet only through lactation (Trial II) were characterized by a higher number of liveborn piglets ($P \leq 0.05$) than sows from the control group. The birth weight of piglets from group E-30G sows was higher compared with that of piglets from the control and E-30L groups. Similarly as the liveborn piglets, on

the weaning day the E-30G sows had the largest litters. Piglet weaning weight in Trial II did not differ significantly among the groups and the same holds true for weight gain and feed intake. Piglet mortality rates during the lactation period were the lowest in the group with the LPC dietary additive supplied at lactation: 4.6%. This value proved to be significantly lower compared with the control and E-30G groups (6.3% and 6.1%, respectively).

The results concerning the period after piglet weaning to day 84 of age were close in Trials I and II regarding final body weight, piglet daily weight gains, feed intake and feed conversion. Statistically significant differences were found only for piglet mortality rate. Similarly to the lactation period, lower piglet mortality in Trial I was noted in groups E-15 and E-30 as compared with the control. The same relation was observed for diarrhoea mortality when the lowest losses occurred in the experimental groups (E-15, 3.5% and E-30, 3.4%). In Trial II, lower piglet mortality was noted only for those from E-30L sows; it was significantly lower (8.1%) compared with the control (10.7%) and E-30G (11.3%) groups.

Blood indices of sows in gestation

Table 5 summarizes the haematological and biochemical blood indices of sows during gestation. The red blood cell count (RBC) in Trial I was highest in group E-30. In Trial II, however, the E-30G sows has a higher RBC count, while in group E-30L the red blood cell concentration did not differ from that in control sows. A higher haemoglobin (Hb) level than in the controls was found in the sows from groups E-15 and E-30 (Trial I) and E-30G (Trial II). The haematocrit (Ht) in Trial I was the highest (P ≤ 0.05) for the sows from group E-30, whereas in Trial II, from E-30G. A significantly higher WBC count in Trial I was determined in the blood samples obtained from the sows receiving 1.5% lucerne preparation in their diet, while in Trial II, no statistically significant differences for this index were noted. The continuous LPC supplementation contributed to a significantly elevated total protein (TP) content in blood in groups E-15 and E-30, as well as in E-30G (Trial II). No significant differences were found among the groups in glucose and triglycerides in both dietary treatments. In Trial I, the lowest uric acid content (UA) was in blood of E-15 sows, while the lowest urea level (BUN) in group E-30. In Trial II, only for uric acid, the lowest contents were recorded in group E-30G. Statistically significant differences in lactic dehydrogenase (LDH) activity, were found only in Trial I, importantly, the activity was highest in group E-15. Statistically significant differences were also found in the activity of ALP and ALT (Table 6).

Table 5. Growth performance of piglets fed a mixture with LPC addition

Indices	Trial I			Trial II			SEM [5]
	control	E-15[1]	E-30[2]	control	E-30G [3]	E-30L [4]	
Average No. of piglets							
born alive	11.87[a]	12.14[ab]	12.27[b]	11.75[a]	12.20[b]	11.90[ab]	0.12
weaned in 28 d	11.33[a]	11.78[b]	11.93[b]	11.01[a]	11.45[b]	11.35[ab]	0.11
Total number of piglets							
born alive	237	243	245	235	244	238	1.71
at 28 d	226	236	239	220	229	227	2.82
at 84 d	216	229	232	210	217	219	3.46
Losses of piglets, %							
to 28 d	4.64[b]	2.88[a]	2.45[a]	6.30[b]	6.15[b]	4.62[a]	0.42
to 84 d	8.81[b]	5.83[a]	5.26[a]	10.75[b]	11.27[b]	8.08[a]	0.94
caused by diarrhoea	7.15[b]	3.49[a]	3.43[a]	8.02[b]	7.84[b]	5.56[a]	1.11
Body weight, kg							
at birth	1.64[a]	1.73[b]	1.75[b]	1.62[a]	1.72[b]	1.61[a]	0.02
at 28 d	7.29	7.43	7.39	7.18	7.21	7.39	0.18
at 84 d	30.12	30.95	30.59	29.47	30.22	30.52	0.31
Average daily gains, g · d^{-1}							
1–28 d	208	211	209	199	196	206	8.2
29–84 d	408	420	414	398	411	413	20.3
Feed intake, kg							
1–28 d	5.07[b]	4.92[ab]	4.82[a]	5.09	5.06	5.08	0.04
29–84 d	828	836	831	835	837	829	10.4
Feed conversion ratio, kg · kg^{-1}							
29–84 d	2.03	1.99	2.01	2.10	2.04	2.01	0.07

[1–5] see Tables 2 and 4; [a,b] - values in experiment I or II in the same rows with different letters differ significantly (*P* ≤ 0.05)

Table 6. Blood parameters of sows during gestation

Indices	Trial I			Trial II			SEM [5]
	control	E-15[1]	E-30[2]	control	E-30G[3]	E-30L[4]	
Haematological indices							
RBC, $10^{12} \cdot l^{-1}$	5.92ᵃ	6.23ᵃᵇ	6.55ᵇ	5.98ᵃ	6.51ᵇ	5.94ᵃ	0.059
Hb, mmol $\cdot l^{-1}$	6.46ᵃ	7.07ᵇ	7.15ᵇ	6.42ᵃ	7.19ᵇ	6.48ᵃ	0.079
Ht, %	37.12ᵃ	38.29ᵃᵇ	39.84ᵇ	36.71ᵃ	39.18ᵇ	36.86ᵃ	0.375
WBC, $10^{9} \cdot l^{-1}$	15.43ᵃᵇ	16.46ᵇ	14.36ᵃ	15.24	14.11	15.29	0.338
GRA, %	44.03ᴬ	49.44ᴬᴮ	54.24ᴮ	44.58ᴬ	53.51ᴮ	45.46ᴬ	1.68
LYM, %	51.15ᴮ	46.97ᴮ	40.69ᴬ	50.64ᴮ	42.92ᴬ	49.87ᴮ	1.26
MID, %	4.82	5.08	3.59	4.78	3.57	4.67	0.321
Biochemical indices							
TP, g $\cdot l^{-1}$	89.45ᵃ	97.36ᵇ	97.00ᵇ	89.24ᵃ	97.43ᵇ	89.78ᵃ	0.903
GLU, mmol $\cdot l^{-1}$	5.54	5.49	5.57	5.42	5.53	5.48	0.046
TG, mmol $\cdot l^{-1}$	1.10	1.02	1.04	1.14	1.17	1.09	0.010
UA, mmol $\cdot l^{-1}$	1.43ᵇ	1.31ᵃᵇ	1.23ᵃ	1.48ᵇ	1.21ᵃ	1.45ᵇ	0.019
BUN, mmol $\cdot l^{-1}$	8.68ᵇ	7.16ᵃ	7.79ᵃᵇ	8.84	7.95	8.73	0.211
LDH, U $\cdot l^{-1}$	673ᵃ	751ᵇ	681ᵃ	687	698	676	5.81
ALP, U $\cdot l^{-1}$	149.2ᴮ	87.5ᴬ	87.0ᴬ	153.7ᴮ	93.4ᴬ	147.9ᴮ	6.21
ALT, U $\cdot l^{-1}$	52.2ᵇ	46.1ᵃ	50.1ᵃᵇ	54.7ᵇ	47.4ᵃ	55.8ᵇ	0.891
AST, U $\cdot l^{-1}$	22.21	23.08	22.74	23.17	24.71	23.83	0.538

[1–5] see Table 4; RBC – red blood cells, Hb – haemoglobin concentration, Ht – haematocrit, WBC – white cell blood cell count, LYM – lymphocytes, MID – middle-sized cells, GRA – granulocytes, TP – total protein, GLU – glucose, TG –triacylglycerols UA – uric acid, BUN – blood urea nitrogen, LDH – lactate dehydrogenase, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase; [AB,ab] – values in experiment I or II in the same rows with different letters differ significantly at $P \le 0.01$ and $P \le 0.05$, respectively

Blood indices of sows in lactation

The haematological and biochemical blood indices of lactating sows are summarized in Table 7. Significantly higher ($P \le 0.05$) RBC counts were found in the blood of the sows from groups E-15 and E-30 in Trial I and E-30L in Trial II. Both the sows fed the dietary additive at a dose of 15 and 30 g LPC in Trial I and 3.0% LPC only during lactation showed higher blood haemoglobin contents as compared with the control. The sows from group E-15 in Trial I were characterized by the highest Ht, while in Trial II, the highest value of this index was

Table 7. Blood parameters of sows during lactation

Indices	Trial I			Trial II			SEM[5]
	control	E-15[1]	E-30[2]	control	E-30G[3]	E-30L[4]	
Haematological indices							
RBC, $10^{12} \cdot l^{-1}$ [6]	6.28ᵃ	7.39ᶜ	7.12ᵇ	6.31ᵃ	6.38ᵃ	7.24ᵇ	0.067
Hb, mmol $\cdot l^{-1}$ [7]	5.83ᵃ	6.70ᵇ	6.74ᵇ	5.89ᵃ	6.03ᵃ	6.87ᵇ	0.097
Ht, % [8]	23.35ᵃ	31.59ᶜ	28.48ᵇ	22.59ᵃ	23.86ᵃ	27.84ᵇ	0.633
WBC, $10^{9} \cdot l^{-1}$ [9]	11.78ᴮ	11.78ᴮ	8.71ᴬ	10.89ᴮ	11.84ᴬᴮ	9.57ᴬ	0.319
GRA, % [10]	47.74ᵃ	55.03ᵇ	59.96ᵇ	48.17ᵃ	49.21ᵃ	58.19ᵇ	1.334
LYM, % [11]	48.07ᶜ	41.36ᵇ	35.57ᵃ	48.54ᵇ	47.23ᵇ	38.25ᵃ	1.329
MID, % [12]	4.19	3.61	4.47	3.29	3.56	3.66	0.182
Biochemical indices							
TP, g $\cdot l^{-1}$ [13]	64.46ᴬ	70.9ᴮ	70.72ᴮ	64.14ᵃ	65.87ᵃ	68.53ᵇ	0.742
GLU, mmol $\cdot l^{-1}$ [14]	4.95	4.98	4.96	4.89	4,93	4.95	0.013
TG, mmol $\cdot l^{-1}$ [15]	0.65ᵃ	0.64ᵃ	0.72ᵇ	0.68	0.69	0.71	0.014
UA, mmol $\cdot l^{-1}$ [16]	0.19	0.15	0.17	0.18	0.17	0.16	0.011
BUN, mmol $\cdot l^{-1}$ [17]	10.48ᵇ	7.10ᵃ	7.17ᵃ	10.56ᵇ	7.69ᵃ	7.12ᵃ	0.412
LDH, U $\cdot l^{-1}$ [18]	690.6ᵃ	764.9ᵇ	717.4ᵃ	676.3ᵃ	682.4ᵃᵇ	725.8ᵇ	10.85
ALP, U $\cdot l^{-1}$ [19]	136.57ᴮ	89.56ᴬ	84.13ᴬ	139.2ᶜ	116.5ᴮ	89.45ᴬ	7.09
ALT, U $\cdot l^{-1}$ [20]	53.54ᵇ	44.20ᵃ	47.17ᵃᵇ	51.47ᵇ	49.56ᵃᵇ	46.76ᵃ	4.92
AST, U $\cdot l^{-1}$ [21]	19.93ᵃ	23.32ᵇ	21.83ᵃᵇ	19.14ᵃ	21.11ᵃᵇ	22.83ᵇ	1.42

[1–5] see Tables 2 and 4; [6–21] see Table 6; [ABC,ab] – values in experiment I or II in the same rows with different letters differ significantly at $P \le 0.01$ and $P \le 0.05$, respectively

recorded in the E-30L group. Including lucerne in lactating sow diets significantly decreased the WBC level in sow blood. A significantly higher protein level (TP) in blood was noted in groups E-15, E-30 and E-30L as compared with the control. Only in Trial I were statistically significant differences observed in the triglyceride level among the sows from group E-30 and groups E-15 and C. The uric acid content for all the groups from both dietary treatments was similar, while the urea content varied between the groups and its highest level was detected in the blood of control sows. LDH activity in Trial I in group E-30 was significantly higher than in groups E-15 and C. In Trial II, only in group E-30L was higher LDH activity noted compared with the control. Incorporating LPC into sow feed significantly ($P \leq 0.01$) reduced ALP activity. It was found that LPC dietary supplementation weakened ALT activity as compared with the control; in Trial I this effect was confirmed in group E-15, whereas in Trial II, in E-30L.

Analysis of the blood haematological and biochemical parameters of 28-day-old piglets is presented in Table 8. Trial I revealed a stronger effect of the investigated feed additive on modification of haematological indices than Trial II. In both groups, i.e. E-15 and E-30, highly significant differences were reported in haematocrit levels against the control. Only in E-30, the supplement under study contributed to increased WBC count ($P \leq 0.05$). In addition, some significant changes were noticed in the granulocyte and lymphocyte numbers in the groups with the LPC dietary additive. Introduction of 3.0% LPC into the diet reduced the glucose level in piglet blood. The triglyceride level in Trial I was significantly higher in group E-15 as compared with the control and E-30. Despite the significant differences in uric acid (UA) levels among the groups in Trials I and II, the investigated preparation was not found to affect the BUN level. The diet including lucerne preparation did, however, significantly reduced the activity of the studied enzymes (ALP, ALT, AST).

Blood indices of weaner piglets

The LPC-containing diets for piglets improved the erythrocyte system, increased the RBC count, haemoglobin level and haematocrit value, especially in groups E-30 and E-30L compared with the control. The LPC feed supplement promoted a decrease in the WBC count in piglet blood. Both the 1.5% and 3.0% LPC additives to piglet diets were conducive to increasing TP, but in Trial II, only in group E-30L. The piglets from groups E-15 and E-30L had the lowest blood triglyceride levels. The urea content in piglet blood throughout Trial I was highest in group E-30L and exceeded that in the control group by 0.84 mmol · l^{-1}. The activity of each enzyme in the experimental groups differed significantly as compared with the control group. Lactic dehydrogenase (LDH) activity was lower in response to feeding the experimental factor by 43% and 34%, respectively, in groups E-15 and E-30, whereas in

Table 8. Blood parameters of weaned piglets at 28 days of life

Indices	Trial I			Trial II			SEM [5]
	control	E-15[1]	E-30[2]	control	E-30G[3]	E-30L[4]	
Haematological indices							
RBC, 10^{12} · l^{-1} [6]	7.41	7.52	7.57	7.46	7.53	7.55	0.07
Hb, mmol · l^{-1} [7]	7.65	7.58	7.67	7.61	7.57	7.62	0.08
Ht, % [8]	41.32[B]	37.61[A]	38.12[A]	39.83	37.65	38.02	0.48
WBC, 10^9 · l^{-1} [9]	11.85[a]	12.27[ab]	13.23[b]	11.89	12.04	12.62	0.25
GRA, % [10]	34.63[a]	40.85[b]	38.76[b]	36.62[a]	36.98[a]	38.73[b]	1.21
LYM, % [11]	63.99[b]	57.53[a]	59.66[a]	61.94[b]	61.41[b]	59.69[a]	1.12
MID, % [12]	1.38	1.62	1.58	1.44	1.61	1.58	0.42
Biochemical indices							
TP, g · l^{-1} [13]	54.01	55.97	55.97	54.23	55.02	55.93	0.74
GLU, mmol · l^{-1} [14]	6.74[b]	6.23[ab]	5.77[a]	6.53[b]	6.38[b]	5.79[a]	0.12
TG, mmol · l^{-1} [15]	0.32[a]	0.45[b]	0.28[a]	0.37[ab]	0.42[b]	0.32[a]	0.02
UA, mmol · l^{-1} [16]	0.15[a]	0.29[b]	0.12[a]	0.17[ab]	0.21[b]	0.12[a]	0.021
BUN, mmol · l^{-1} [17]	6.51	7.18	7.24	6.85	6.52	6.34	0.99
LDH, U · l^{-1} [18]	712.9	681.9	781.7	723.4	708.9	742.3	32.36
ALP, U · l^{-1} [19]	227.2[A]	258.9[B]	261.2[B]	223.4[A]	253.5[B]	264.1[B]	8.13
ALT, U · l^{-1} [20]	23.71[A]	30.72[B]	32.40[B]	23.42[A]	26.25[AB]	31.35[B]	0.91
AST, U · l^{-1} [21]	50.21[a]	57.03[b]	58.45[b]	50.2[a]	56.39[b]	58.07[b]	1.69

[1–5] see Table 4; ; [6–21] see Table 6; [ABC,ab] – values in experiment I or II in the same rows with different letters differ significantly at $P \leq 0.01$ and $P \leq 0.05$, respectively

Table 9. Blood parameters of piglets at 80 days of life

Indices	Trial I			Trial II			SEM[5]
	control	E-15[1]	E-30[2]	control	E-30G[3]	E-30L[4]	
Haematological indices							
RBC, $10^{12} \cdot l^{-1}$ [6]	6.56[a]	7.41[b]	7.97[b]	6.39[a]	6.88[ab]	7.72[b]	0.167
Hb, mmol $\cdot l^{-1}$ [7]	6.31[a]	6.47[ab]	6.77[b]	6.28[a]	6.53[ab]	6.71[b]	0.097
Ht, % [8]	34.33[a]	37.82[b]	38.12[b]	32.94[a]	33.86[b]	34.85[b]	0.633
WBC, $10^9 \cdot l^{-1}$ [9]	16.13[b]	15.35[ab]	14.23[a]	15.94[c]	14.64[b]	13.57[a]	0.319
GRA, % [10]	55.64[b]	57.66[b]	48.76[a]	56.13[b]	55.22[b]	50.19[a]	1.334
LYM, % [11]	43.03[b]	40.23[a]	49.36[c]	42.55[a]	43.23[a]	48.25[b]	1.329
MID, % [12]	1.33	2.11	1.88	1.32	1.55	1.56	0.182
Biochemical indices							
TP, g $\cdot l^{-1}$ [13]	53.71[A]	57.89[B]	63.17[C]	54.17[a]	55.83[a]	62.54[b]	0.742
GLU, mmol $\cdot l^{-1}$ [14]	6.26	6.03	6.28	6.07	5.93	6.15	0.013
TG, mmol $\cdot l^{-1}$ [15]	0.77[b]	0.55[a]	0.61[ab]	0.78[b]	0.69[ab]	0.62[a]	0.014
UA, mmol $\cdot l^{-1}$ [16]	0.33	0.34	0.33	0.38	0.37	0.34	0.011
BUN, mmol $\cdot l^{-1}$ [17]	6.45[a]	6.66[a]	7.29[b]	6.54[a]	6.76[ab]	7.12[b]	0.123
LDH, U $\cdot l^{-1}$ [18]	987.6[b]	689.7[a]	737.6[a]	897.5[b]	681.3[a]	765.8[ab]	21.85
ALP, U $\cdot l^{-1}$ [19]	225.6[B]	278.7[A]	298.8[A]	239.2[a]	246.5[ab]	279.4[b]	7.09
ALT, U $\cdot l^{-1}$ [20]	21.05[a]	21.82[a]	30.01[b]	21.44[a]	24.53[ab]	28.76[b]	0.92
AST, U $\cdot l^{-1}$ [21]	53.56[a]	54.13[a]	60.95[b]	49.17[a]	51.11[a]	62.83[b]	1.42

[1–5] see Table 4; [6–21] see Table 6; [ABC,ab] – values in experiment I or II in the same rows with different letters differ significantly at $P \leq 0.01$ and $P \leq 0.05$, respectively

Trial II, a statistically significant difference was reported only in group E-30G. Regarding ALP, highly significant elevation of activity was observed in Trial I, i.e. by 23% and 32%, respectively, in groups E-15 and E-30, while in Trial II, only in group E-30L. In Trial I, the ALT activity increased by 42% as a result of including 3.0% LPC in sow diets. In Trial II, a significant rise of ALT activity (by 34%) was found in group E-30L. AST activity also depended on the studied feed additive. It was found that in group E-30 the activity of ALP increased by 14%, whereas in E-30L, by as much as 28% compared with the control.

Discussion

Application of plant-derived feed additives positively affects daily weight gains, feed intake and feed conversion ratio (Wang et al., 2008). This correlation was observed in the present studies, 15 and 30 g of LPC preparation per kilogram of feed promoted the growth of sow body weight in late gestation (Trial I), whereas in the groups fed LPC periodically, only the sows with the lucerne preparation supplied during gestation had a higher prepartum body weight. Both periodic use of the LPC preparation (during lactation) and continuous use (through the whole reproductive period) at the 3.0% level allowed for higher energy reserves in the body, which ensured better body condition of sows after lactation. Dietary incorporation of LPC increased piglet

birth weight and the number of liveborn ones, especially at 3.0% during gestation ($P \leq 0.05$). Opposing relationships were shown by Lian et al. (2004) in a study in which lucerne meal added in an amount of 20% to diets for sows in gestation had no significant effect on born litter weight or piglet weight gain, but significantly increased litter weight and piglet weight gain of weaned piglets. Phytoestrogens (coumestrol, genistein, daidzein) found in lucerne were likely to elevate piglet birth weight. The finding was confirmed by studies on sows that received a dietary supplement of daidzein in late gestation (Li et al., 1999; Ren et al., 2001). Ren et al. (2001) hypothesized that the foetal growth-promoting effect of daidzein is *via* activation of insulin-like growth factor (IGF-1). The preweaning piglet mortality rate depends on several factors, among others, birth weight, litter size, length of farrowing, sow milk production, structure of pens, ambient temperature and behaviour patterns of sows and piglets (Panzardi et al., 2013). In the groups fed the LPC-supplemented diet, the mortality rates of piglets up to 28 days of life were lower compared with the control. A similar relationship was highlighted in other works studying the effect of various phytobiotics on piglet rearing performance (Walter and Bilkei, 2004; Matysiak et al., 2012). Matysiak et al. (2012) reported that dietary inclusion of a phytobiotic reduced piglet mortality during lactation from 8.6% in the control to 4.5% in the experimental group. Importantly, in Trial I the mortality

rate caused by diarrhoea was two-fold lower in the experimental groups than in the control. The active substances of herbs and some plants enhance enzyme secretion, boost the immune system and prevent diarrhoea (Matysiak et al., 2012). Lucerne saponins possess potent antibacterial properties (Avato et al., 2006; Joy and George, 2014) that contribute to the declining incidence of diarrhoea induced by *E. coli* in the postweaning period.

The red and white blood cell counts, haemoglobin concentration, and haematocrit value in blood of piglets and control sows indicated proper erythropoiesis and the obtained values were within the reference ranges (Friendship and Henry, 1996; Winnicka, 2004). The present study shows a significant impact of LPC on the haematological parameters of sow and piglet blood, especially the red blood cell count (RBC) and haemoglobin level (Hb). Some authors (Vyas et al., 2010; Davys et al., 2011) attribute that to the high level of iron in LPC and to this iron being readily absorbed and incorporated into human and animal red blood cells. In a study by Criste et al. (2008), 5.0% dietary lucerne given to weaned piglets significantly influenced iron balance, however, studies on turkeys (Krauze and Grela, 2010) did not confirm the effectiveness of LPC in changing RBC indices. Vyas et al. (2010) in studies on humans associated the increased level of haemoglobin and iron in the blood collected from the experimental group with the rich mineral composition of the lucerne preparation. This pertains predominantly to Fe and Cu, which are directly involved in haematopoiesis, but the presence of vitamin A and β-carotene enhances the absorption of these elements. However, no significant influence of LPC on the level of RBC and Hb was noted in the case of 28-day-old pigs, as unweaned piglets exhibit low intake of solid feed and colostrum and sow`s milk are a rather poor source of Fe (Hanušovský et al., 2014). A high content of biologically active compounds in LPC, e.g., saponins and daidzein (EFSA, 2009; Grela and Pietrzak, 2014), was likely to cause statistically significant differences in the WBC and LEU counts, notably as a result of the immunomodulatory effects of saponins, among others, *via* antibody and cytokinin production (Francis et al., 2002; Das et al., 2012). Our study found a decline in white blood cell and leucocyte counts in the experimental groups of both sows and piglets. Wang et al. (2002) found that multiple injections of 0.5 mg daidzein per kilogram BW for 24 days into piglets increased WBC and LEU counts; also in other studies Wang (2007) shows the same immunostimulatory, in particular, effect of lucerne saponins on pigs and improvement in the WBC count

when lucerne saponins were added at a dose of 0.5% to the pig diets. A difference between the results of the present studies may arise from the synergistic activity of a number of other biologically active substances that LPC contains. Moreover, the route of administration of an experimental factor (injection or feeding) also influences the obtained results. The activity of enzymes from the aminotransferase group and alkaline phosphatase points to the effect of the biologically active ingredients of LPC on the metabolism of some tissues and organs (e.g., the liver) of sows and piglets. The finding is consistent with the studies on fatteners conducted by Grela et al. (2008) and Pietrzak and Grela (2013), which demonstrated a significantly higher liver weight of fatteners fed 1.5% and 3.0% LPC. These authors associate the increased liver weight with the influence of bioactive compounds in the lucerne preparation. AST and ALT are enzymes recognized as indices of protein reserve mobilization in an organism in the case of a negative energy balance, while limited activity of these enzymes in the sows fed LPC-supplemented feed indicates better utilization of the protein and energy in the feed. The studies of Yang et al. (2002) also showed a significant influence of lucerne on AST activity. A different relationship was noted for alkaline phosphatase activity in piglets. ALP is involved in phosphate metabolism and the enhanced activity of this enzyme occurs mainly during growth of the skeletal system growth and during development. The LPC preparation is high in Ca and P, which could favour a rise in ALP activity.

Conclusions

The results of the present study indicate that the addition of 1.5% or 3.0% of lucerne protein concentrate (LPC) to sow and piglet diets improves erythropoiesis, as manifested by the elevated red blood cell indices. The biologically active compounds contained in LPC contributed to the better performance results of sows and their piglets. Further investigations are necessary to better clarify the mechanisms involved in the effects of LPC on animal organisms. It seems that the continuous inclusion of 3.0% LPC in sow diets can be recommended and that for piglets, LPC in amount of 1.5% can be added to starter diets in the postweaning period.

Acknowledgements

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