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**Nutrient digestibility and nitrogen balance
in growing-finishing pigs fed legume-based diets**

Strawność składników pokarmowych i bilans azotu u tuczników żywionych
dawkami z udziałem krajowych nasion roślin strączkowych

Summary. Four experiments were conducted to determine the effect of replacing soybean meal with pea (two cultivars), faba bean or yellow lupine seeds in pig diets supplemented or not supplemented with feed enzymes on nitrogen balance and nutrient digestibility. The experimental materials comprised 72 hybrid (Polish Large White × Polish Landrace) growing-finishing pigs. Balance and digestibility tests were carried out twice (at the first and second stages of fattening), by the simple balance method. At the first stage of fattening, soybean meal was partially replaced with pea (40% or 46%), faba bean (33%) or yellow lupine (18%) seeds. At the second stage of fattening, soybean meal was completely replaced with legume seeds. Nutrient digestibility from PT-1 diets containing pea seeds of both cultivars was comparable with that noted for standard diets, or even higher. A 33% faba bean content of experimental diets was too high, and it decreased the digestibility of all nutrients. Enzyme supplementation reduced the adverse effect of faba bean, yet nutrient digestibility did not reach the level reported for the control group. A significant increase in nutrient digestibility was noted in pigs fed diets containing yellow lupine seeds, and the beneficial influence of lupine was enhanced by enzyme supplementation. At the second stage of fattening, nutrient digestibility increased when yellow lupine seeds were used as a substitute for soybean meal. The complete replacement of soybean meal with pea and faba seeds in enzyme-supplemented diets also positively affected nutrient digestibility. Soybean meal substitutes had no negative impact on nitrogen balance at both stages of fattening. A two-factorial analysis of variance (with legume species and dietary enzyme supplementation as experimental factors) showed that pea and yellow lupine seeds are suitable substitutes for soybean meal in pig diets.

Key words: pea, faba bean, yellow lupine, enzymes, pigs, digestibility, N balance

INTRODUCTION

An analysis of the use of high-protein feed materials shows that soybean meal has been the unquestioned leader on the animal feed market for years. The dominant position of soybean meal, resulting from the ban on the use of animal meals and genetically modified feed materials, has shifted the interest of animal producers to alternative sources of protein, including locally available high-protein components. The increasing popularity of organic pig farming and the growing consumer demand for high-quality pork products are also important considerations.

The benefits of grain legumes [Rochester *et al.* 1998, Evans *et al.* 2001], including for sustainable agriculture [Poetsch 2006, Badgley *et al.* 2007], and the fact that their yield potential has not been fully exploited to date, have spurred interest in this group of feed materials. Research results confirm the high nutritional value of pea, faba bean and lupine seeds [Crépon *et al.* 2009, Jezierny *et al.* 2010], and their importance as protein and energy sources [Bach Knudsen 1997, Salgado *et al.* 2002]. Moreover, many problems resulting from the presence of anti-nutritional compounds in legume seeds have been solved through the breeding of varieties with a lower content of harmful substances [Champ 2002]. In view of the above, the norms and standards regarding the legume content of pig diets should probably be revised.

The objective of this study was to determine the effect of replacing soybean meal with legume seeds as high-protein components in pig diets on nutrient digestibility and nitrogen balance. The benefits of exogenous enzyme dietary supplementation were also estimated.

MATERIALS AND METHODS

The seeds of two pea cultivars differing in protein concentration (Mazurek and Piast), faba bean seeds (cv. Nadwiślański) and yellow lupine seeds (cv. Juno) were used in the study. Four growth trials (30 kg to 100 kg BW), involving 72 pigs (Polish Large White × Polish Landrace), were carried out under laboratory conditions at the Department of Animal Nutrition and Feed Science, University of Warmia and Mazury in Olsztyn (Purwin, Stanek 2010). At the first stage of fattening, soybean meal was partially replaced with pea (40% or 46%), faba bean (33%) or yellow lupine (18%) seeds, in accordance with the pig nutrient requirements [Normy... 1993]. In each trial, legume-based diets were supplemented or not with the same exogenous enzymes (cellulase, β -glucanase, pentosanase, hemicellulase, pectinase and β -xylanase).

At the second stage of fattening, soybean meal was completely replaced with legume seeds (tab. 1). Nitrogen balance and nutrient digestibility tests were performed by the simple balance method, twice during each trial, at approximately 60 kg and 80 kg BW. Each experimental group comprised six animals kept in individual cages. In each digestibility test, the experimental period proper lasted seven days.

The effects of legume species and dietary enzyme supplementation on nutrient digestibility and nitrogen balance were also determined.

All data were analyzed statistically by two-way ANOVA and Duncan's multiple range test, using Statistica 7.1 software (StatSoft Inc. 2006).

Table 1. Composition and nutritive value of diets
Tabela 1. Skład i wartość pokarmowa mieszanek

Diets for growing pigs – Mieszanki PT-1												
Components, % Składniki, %	pea var. mazurek groch odm. mazurek			pea var. piast groch odm. piast			faba bean bobik			yellow lupine lubin żółty		
	C K	P G	P+E G+E	C K	P G	P+E G+E	C K	FB B	FB+E B+E	C K	YL ŁŻ	YL+E ŁŻ+E
Barley ground Śruta jęczmienna	78.50	51.44	51.39	79.35	45.35	45.30	78.25	60.84	60.76	78.26	74.26	74.14
Soya bean meal Poekstrakcyjna śruta sojowa	19.00	6.00	6.00	18.00	6.00	6.00	19.00	3.50	3.50	19.00	5.00	5.00
Pea var. Mazurek Groch odm. Mazurek	-	40.00	40.00	-	-	-	-	-	-	-	-	-
Pea var. Piast Groch odm. Piast	-	-	-	-	46.00	46.00	-	-	-	-	-	-
Faba bean Bobik	-	-	-	-	-	-	-	33.00	33.00	-	-	-
Yellow lupine Łubin żółty	-	-	-	-	-	-	-	-	-	-	18.00	18.00
Feed additives ¹ Dodatki paszowe ¹	2.50	2.56	2.61	2.65	2.65	2.70	2.75	2.66	2.74	2.74	2.74	2.86
ME, MJ/kg EM, MJ/kg	12.59	12.89	12.97	12.87	13.05	13.20	12.73	12.04	12.44	12.35	12.45	12.90
Crude protein, g Białko ogólne, g	161.3	163.9	163.2	165.5	166.7	166.9	166.5	163.4	164.9	168.3	168.5	164.7
Crude fiber, g Włókno surowe, g	35.1	43.9	44.1	40.4	45.0	45.1	41.2	47.9	48.3	41.5	58.8	57.8
Diets for finishing pigs – Mieszanki PT-2												
Barley ground Śruta jęczmienna	79.50	52.44	52.39	79.50	51.60	51.55	81.48	62.44	62.35	83.39	79.34	79.24
Soya bean meal Poekstrakcyjna śruta sojowa	18.00	-	-	18.00	-	-	16.00	-	-	14.00	-	-
Pea var. Mazurek Groch odm. Mazurek	-	45.00	45.00	-	-	-	-	-	-	-	-	-
Pea var. Piast Groch odm. Piast	-	-	-	-	46.00	46.00	-	-	-	-	-	-
Faba bean Bobik	-	-	-	-	-	-	-	35.00	35.00	-	-	-
Yellow lupine Łubin żółty	-	-	-	-	-	-	-	-	-	-	18.00	18.00
Feed additives ¹ Dodatki paszowe ¹	2.50	2.56	2.61	2.50	2.40	2.45	2.52	2.56	2.65	2.61	2.66	2.76
ME, MJ/kg EM, MJ/kg	13.27	13.53	13.70	12.84	13.20	13.38	12.98	12.44	12.77	12.98	12.67	12.65
Crude protein, g Białko ogólne, g	145.4	151.1	150.2	14.88	14.86	14.86	155.8	155.9	155.1	155.8	148.2	148.0
Crude fiber, g Włókno surowe, g	37.3	42.1	41.7	44.0	47.3	47.8	41.5	49.7	49.8	41.5	65.8	64.9

¹ Limestone, dicalcium phosphate, NaCl, mineral-vitamin premix, enzymes

¹ Kreda pastewna, fosforan dwuwapniowy, NaCl, premix mineralno-witaminowy, enzymy

RESULTS AND DISCUSSION

Nutrient digestibility levels were significantly affected by the source of dietary protein (tab. 2). The seeds of both pea cultivars, added to PT-1 diets as a partial substitute for soybean meal, contributed to high protein digestibility, at 76–79%, which was only slightly below the values reported by Mariscal-Landin *et al.* [2002]. Pea seeds had a beneficial influence also on the digestibility of other nutrients. A positive effect of replacing half of the soybean meal included in the diet with pea seeds has been observed by Thacker and Racz [2001]. The results of an experiment conducted by Hanczakowska and Świątkiewicz [2008] indicate the possibility of partial replacement of soybean meal with pea seeds, whereas an earlier study by Hanczakowska and Urbańczyk [2001] suggests that the inclusion of pea seeds in pig diets could decrease protein digestibility.

Dietary enzyme supplementation did not increase feed efficiency, which is consistent with the findings of Castella and Cliplefa [1992], Stanek [1999], Thacker and Racz [2001]. According to Bach Knudsen [1997] and Baucells *et al.* [2000], enzymes may positively affect feed conversion in growing-finishing pigs fed pea-based diets.

A 33% faba bean content of experimental diets was too high, and it highly significantly decreased the digestibility of all nutrients. As demonstrated by O'Doherty and Keady [2001], and Urbańczyk [2001], the inclusion of up to 20% faba bean in pig diets does not lead to a drop in production levels. Larger amounts of faba bean seeds, containing non-starch polysaccharides and tannins, may lower nutrient digestibility and availability [Gdala *et al.* 1995, Gdala and Buraczewska 1997]. According to Crépon *et al.* [2009], a 35% faba bean content of pig diets provides feed efficiency comparable to that reported for soybean meal. As suggested by Bedford [1995, 2000], and Bedford and Schulze [1998], enzymes modify the structure of non-starch polysaccharide fibers in legumes and minimize their adverse effects. In our study, enzyme supplementation reduced the negative effect of faba bean, yet nutrient digestibility did not reach the level reported for the control group.

A significant increase in nutrient digestibility, except for crude fiber (39.3% vs. 35.4%), was noted in pigs fed diets containing yellow lupine seeds (18%). Similar digestibility of protein from lupine seeds, resulting from high intestinal absorption (82.1% to 89.4%) [Mariscal-Landin *et al.* 2002], has been observed by Kasproicz and Frankiewicz [2001]. The beneficial influence of lupine was enhanced by enzyme supplementation, and nutrient digestibility exceeded the levels noted in the control group. It seems that feed enzymes affecting the carbohydrate fractions supported also the digestion of other nutrient, including crude protein.

A beneficial influence of enzymes added to yellow lupine-based diets has been reported by Gdala *et al.* [1995], Flis *et al.* [1998] and Jezierny *et al.* [2010], who observed a significant increase in α -galactoside digestibility in the small intestine of growing pigs, as well as higher digestibility levels of N-free extracts, amino acids and energy. According to Van Nevel [2000], the effectiveness of lupine-enriched diets is determined by the applied variety of lupine and its percentage content.

At the second stage of fattening, the response of older animals to soybean meal substitutes was weaker and varied. Pea seeds had a positive effect on nutrient digestibility, but the efficacy of enzymes was low. Older animals were less sensitive to the 35% faba bean content of diets. The digestibility of protein, fiber and energy was slightly lower,

Table 2. Nutrient digestibility, %
Tabela 2. Strawność składników pokarmowych, %

Item Wyszczególnienie	Pea var. Mazurek Groch odm. Mazurek				Pea var. Piast Groch odm. Piast				Faba bean Bobik				Yellow lupine Lubin żółty					
	P		P+EG +E		P		P+EG +E		C		FB		FB+E B+E		YL		YL+E ŁŻ+E	
	C	K	G		C	K	G		C	K	C	B	B	B	C	ŁŻ	K	ŁŻ
Diets for growing pigs – Mieszanki PT-1																		
Crude protein Białko ogólne	77.7	76.2	78.0	78.7	79.1	78.7	78.6	75.5 ^A	67.2 ^{Ba}	71.9 ^{Bb}	73.8 ^B	74.4 ^B	78.9 ^A					
Ether extract Tłuszcz surowy	67.4 ^{Bb}	72.2 ^a	73.5 ^A	63.9 ^{ABa}	67.5 ^A	56.3 ^{Bb}	55.5 ^a	44.2 ^b	53.1 ^a	35.9 ^C	49.9 ^B	63.3 ^A						
Crude fibre Włókno surowe	45.4 ^B	59.8 ^A	59.4 ^A	43.5 ^A	32.4 ^B	44.8 ^A	38.7 ^A	21.2 ^C	25.4 ^B	39.3 ^B	35.4 ^B	44.5 ^A						
N-free extractives Zw. bezazotowe wyciągowe	90.3 ^{Bb}	92.2 ^a	92.6 ^A	91.7 ^a	89.2 ^{Bb}	92.4 ^A	91.6 ^A	90.4 ^B	91.1	87.4	87.5	88.9						
Gross energy Energia brutto	80.3 ^b	83.1 ^a	83.9 ^a	82.9	81.5	83.5	81.7 ^A	77.3 ^{Bb}	79.8 ^a	78.2 ^B	78.3 ^B	80.6 ^A						
Diets for finishing pigs – Mieszanki PT-2																		
Crude protein Białko ogólne	79.5	80.8	83.7	82.2	80.8	81.8	75.0	71.5	77.4	75.2 ^B	76.3 ^B	79.8 ^A						
Ether extract Tłuszcz surowy	42.8 ^B	63.0 ^A	68.9 ^A	49.2 ^B	60.2 ^{ABa}	55.0 ^b	59.0 ^C	62.0 ^B	70.3 ^A	65.0 ^{Bb}	63.5 ^B	68.7 ^A						
Crude fibre Włókno surowe	42.6 ^B	65.0 ^A	69.4 ^A	44.9	41.8 ^b	47.4 ^a	39.2	34.5	34.5	37.6 ^C	46.8 ^B	58.1 ^A						
N-free extractives Zw. bezazotowe wyciągowe	92.9	94.4	94.6	91.6 ^B	89.3 ^C	93.1 ^A	91.4	91.1	91.2	89.9	88.9	89.6						
Gross energy Energia brutto	85.3 ^b	88.3 ^a	88.5 ^a	84.1 ^A	80.0 ^B	85.0 ^A	82.2 ^A	79.7 ^B	82.2 ^A	81.1	79.8 ^B	82.3 ^A						

Means within a row with different superscripts are significantly different: a, b, P ≤ 0.05, A, B, C – P ≤ 0.01
Wartości w rzędach oznaczone różnymi literami różnią się: a, b, P ≤ 0.05, A, B, C – P ≤ 0.01

while enzyme supplementation allowed to achieve digestibility levels equal to or higher than those noted in the control group fed diets with soybean meal as the main protein source.

The digestibility of all nutrients except for crude fat increased when yellow lupine seeds were used as a substitute for soybean meal, in comparison with soybean meal-based diets. A decrease in crude fiber digestibility, noted in younger pigs, was not observed at the second stage of fattening. The quantity of lupine seeds added to PT-2 diets was adequate. Feed enzymes considerably improved the digestibility of all nutrients, particularly protein and crude fiber.

According to Pariza and Cook [2010], diets containing legume seeds should be supplemented with feed enzymes such as α -galactosidase, β -glucanase, hemicellulase, pectinase and xylanase. Enzymatic formulations may exert varied effects [Bedford and Schulze 1998], but in most cases enzymatic supplementation enhances the nutritive value of diets containing legume seeds [Yin *et al.* 2000].

A nitrogen balance analysis (tab. 3) points to certain differences in nitrogen utilization even within legume species, since pea seeds cv. Mazurek (40%) reduced nitrogen retention and utilization. Pea seeds cv. Piast, despite their higher content in experimental diets (46%), had a positive effect on nitrogen balance. Yellow lupine seeds increased nitrogen retention and improved nitrogen utilization. The beneficial influence of yellow lupine seeds and enzyme supplementation on nitrogen balance has been previously noted by Flis *et al.* [1996]. A similar trend regarding nitrogen balance was observed in older animals, at the second stage of the study. An advantageous effect was noted for pea seeds cv. Piast, faba bean seeds and yellow lupine seeds supplemented with enzymes.

Table 3. Nitrogen balance in pigs
Tabela 3. Bilans azotu u świń

Item Wyszczególnienie	Pea var. Mazurek Groch odm. Mazurek			Pea var. Piast Groch odm. Piast			Faba bean Bobik			Yellow lupine Łubin żółty		
	C	P	P+E	C	P	P+E	C	FB	FB+E	C	YL	YL+E
	K	G	G+E	K	G	G+E	K	B	B+E	K	ŁŻ	ŁŻ+E
Diets for growing pigs – Mieszanki PT-1												
N – intake, g N pobrany, g	65.1	65.7	67.3	59.5	59.4	59.5	74.8	74.8	74.4	70.8	66.4	66.1
Retained, g Retencja, g	29.3 ^A	24.0 ^B	22.1 ^B	23.0	23.7	25.0	28.7	30.2	31.2	21.1	23.7	24.6
Retained/intake, % Retencja/pobrany,%	45.0 ^A	36.5 ^B	32.9	38.6	39.9	42.0	38.4	40.4	41.9	29.8	35.7	37.2
Diets for finishing pigs – Mieszanki PT-2												
N – intake, g N pobrany, g	56.8	53.5	57.4	52.9	53.3	53.4	58.6	57.5	58.0	59.3	58.6	58.7
Retained, g Retencja, g	25.8	24.9	23.6	21.7	22.8	22.5	26.1	25.2	26.7	29.6	22.6	27.2
Retained/intake, % Retencja/pobrany,%	45.4	46.5	41.1	41.0	42.8	42.1	44.5	43.8	46.0	49.9	38.6	46.3

Means within a row with different superscripts are significantly different: a, b, $P \leq 0.05$, A, B, C – $P \leq 0.01$
Wartości w rzędach oznaczone różnymi literami różnią się: a, b, $P \leq 0,05$, A, B, C – $P \leq 0,01$

Table 4. Effect of species and enzymes on digestibility and nitrogen balance
Tabela 4. Wpływ gatunku nasion i enzymów na strawność i bilans azotu

Item Wyszczególnienie	Species of seeds – Gatunek nasion			Enzyme – Enzym		SEM
	peas groch	faba bean bobik	yellow lupine łubin żółty	-	+	
Digestibility coefficients – Concentrates for growing pigs						
Crude protein Białko ogólne	77.60 ^A	69.59 ^B	76.83 ^A	74.17	76.65**	0.68
Ether extract Tłuszcz surowy	66.44 ^A	48.63 ^C	56.83 ^B	57.62	61.54	1.54
Crude fibre Włókno surowe	51.72 ^A	23.38 ^B	39.94 ^C	40.00	43.38	1.98
N-free extractives Zw. bezazotowe wyciągowe	92.29 ^A	90.77 ^B	88.46 ^C	90.58	91.33*	0.27
Gross energy Energia brutto	83.23 ^A	78.52 ^B	79.48 ^B	80.40	81.83*	0.45
Digestibility coefficients – Concentrates for finishing pigs						
Crude protein Białko ogólne	82.06	75.83	78.12	78.46	80.58**	0.51
Ether extract Tłuszcz surowy	57.65 ^B	57.78 ^B	66.59 ^A	55.37	64.48**	1.39
Crude fibre Włókno surowe	56.92 ^A	36.78 ^B	52.46 ^A	48.41	53.13	1.80
N-free extractives Zw. bezazotowe wyciągowe	93.50 ^A	90.26 ^{Ba}	89.26 ^{Bb}	91.23	92.03*	0.33
Gross energy Energia brutto	86.48 ^A	80.73 ^B	81.08 ^B	82.97	84.42*	0.51
Nitrogen balance – Concentrates for growing pigs						
Retained, g Retencja, g	23.57 ^{Bb}	25.95 ^A	24.91 ^a	23.93	25.07*	0.18
Retained/intake, % Retencja/pobrano, %	43.20	44.93	42.42	42.85	44.03**	0.56
Nitrogen balance – Concentrates for finishing pigs						
Retained, g Retencja, g	22.86 ^B	30.70 ^A	24.17 ^B	25.41	24.89	0.41
Retained/intake, % Retencja/pobrano, %	37.80 ^b	41.15 ^{Aa}	36.48 ^B	38.12	38.49	0.32

Means within a row with different superscripts are significantly different: a, b, $P \leq 0.05$, A, B, C – $P \leq 0.01$
Wartości w rzędach oznaczone różnymi literami różnią się: a, b, $P \leq 0,05$, A, B, C – $P \leq 0,01$

A two-factorial analysis showed that nutrient digestibility was affected by legume species ($p \leq 0.01$) at the initial stage of fattening. The digestibility of total protein from faba bean was significantly lower, compared with pea and yellow lupine, whereas the digestibility of other nutrients was highest in pigs fed pea-based diets. At the second stage of the study, legume species had no significant effect on protein digestibility, but the digestibility of crude fat, crude fiber, N-free extracts and gross energy was influ-

enced by legume species ($p \leq 0.01, 0.05$). Legume species affected nitrogen retention both at the first and second stage of fattening. The effect of legume species on nitrogen utilization was validated by a statistical analysis only at the final stage. The optimum nitrogen balance values were noted in pigs fed diets containing faba bean.

Regardless of legume species, enzymatic formulations had the most pronounced effect on total protein digestibility at the first and second stage of the study, and on nitrogen utilization at the first stage of fattening ($p \leq 0.01$). Feed enzymes had a beneficial influence ($p \leq 0.05$) on carbohydrate digestibility at both stages of the experiment. The effect of enzyme supplementation on crude fat digestibility increased with age, which is difficult to interpret.

CONCLUSIONS

The results of this study, which investigated the effect of replacing soybean meal with pea, faba bean or yellow lupine seeds in pig diets on nitrogen balance and nutrient digestibility, indicate that legume seeds are suitable substitutes for soybean meal as a protein source in pig diets. The species, variety and dietary inclusion levels of legumes are important considerations. Diets containing legume seeds should be supplemented with enzymes. The use of legumes in animal nutrition remains limited due to their specific structure (carbohydrate fractions, ANF_s), therefore it seems necessary to increase their nutritive value.

In view of our findings and literature data suggesting that legume seeds may be the main or even the only protein source in pig diets, the increased use of legume-based diets, in addition to rapeseed meal, is likely to reduce soybean imports. Thus, the opinion about the relatively low importance of legume seeds as a soybean meal substitute and an alternative protein source need to be reconsidered.

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Streszczenie. W czterech eksperymentach analizowano wpływ zastąpienia śruty poekstrakcyjnej sojowej nasionami grochu (2 odmiany), bobiku lub łubinu żółtego, z dodatkiem enzymów lub bez, na strawność składników pokarmowych i bilans azotu. Na 72 tucznikach (wbc × pbz) metodą bilansową prostą dwukrotnie (I i II okres tuczu) przeprowadzono badanie bilansowo-strawnościowe. W pierwszym okresie tuczu w mieszankach dokonano częściowej substytucji soi, stosując nasiona grochu (40 lub 46%), bobiku (33%) lub łubinu żółtego (18%). W okresie II tuczu całą ilość poekstrakcyjnej śruty sojowej zastąpiono nasionami roślin strączkowych grubonasiennych. Wykorzystanie nasion grochu obu odmian w mieszankach PT-1 zagwarantowało strawność składników pokarmowych jak przy żywieniu standardowym lub nawet nieco ją zwiększyło. Zastosowanie 33% bobiku w mieszance okazało się ilością zbyt dużą, obniżającą strawność wszystkich składników pokarmowych. Dodatek enzymów ograniczył niekorzystny wpływ bobiku, jednak stwierdzona strawność nie osiągnęła poziomu grupy kontrolnej. Istotne zwiększenie strawności obserwowano po podaniu mieszanki z nasionami łubinu żółtego, a ich korzystny wpływ zwiększyła suplementacja enzymatyczna. W II okresie tuczu jednoznacznie korzystnie na strawność wpłynęło całkowite zastąpienie śruty poekstrakcyjnej sojowej nasionami łubinu żółtego, a po uzupełnieniu enzymami grochem i bobikiem. Nie stwierdzono niekorzystnego wpływu zamienników soi na wyniki bilansu azotu w obu okresach. Dwuczynnikowa analiza wariancji uwzględniająca wpływ gatunku nasion oraz dodatku enzymów wskazała na nasiona grochu i łubinu żółtego jako odpowiednie zamienniki soi.

Słowa kluczowe: groch, bobik, łubin żółty, enzymy, tuczniki, strawność, bilans azotu