Effects of Dietary n-3 Rich PUFA Mixture on Performance, Plasma Metabolites and Muscle Fatty Acids Profile in Growing Pigs

Anca Gheorghe*[1], Mihaela Hăbeanu[1], Nicoleta A. Lefter[1], Lavinia Idriceanu[1]

[1]National Research Development Institute for Animal Biology and Nutrition, 077015-Balotesti, Calea Bucuresti, 1, Romania

Abstract

The study was conducted to assess the effect of feeding an n-3 PUFA-enriched diet on growth performance, plasma metabolites, and muscle fatty acids composition in growing pigs. Forty growing pigs (13.1±1.8 kg body weight) were randomly divided to receive a control [C based on corn-triticale-soybean meal (SBM)] or n-3 PUFA mix diet [extruded linseed EL: walnut meal WM, where the 80 g/kg dietary mixture (50:30 *wt/wt*) partially replace SBM] for 30 days. At the end of the trial, blood and muscle samples were collected for plasma profiles and fatty acids analyses. Feeding pigs with n-3 PUFA-enriched diet slightly improved body weight and average daily gain (P>0.05) with similar feed intake, and tend to improve feed efficiency (P=0.058). There was no effect on plasma protein profile, but the plasma urea nitrogen was decreased (P=0.042) indicating a higher utilization of dietary nitrogen. Plasma lipid profile was affected as a response to the dietary mixture by reducing the total cholesterol concentration (P=0.04) vs C diet. The *Longissimus Dorsi* muscle of pigs fed dietary mixture had higher contents in alpha-linolenic acid (P=0.030), docosahexaenoic (P=0.050), total PUFA (P=0.0001) and n-3 PUFA (P=0.019), and a lower n-6: n-3 ratio (P=0.002) vs pigs fed C diet. In conclusion, the dietary mixture had a positive effect on growing pigs' performance and health status, improving the plasma lipo-protein response and enhanced the fatty acid profile of the meat.

Keywords: meat fatty acids, pigs' performance, plasma parameters, n-3 PUFA mix

1. Introduction

Bioactive compounds (e.g. alpha-linolenic acid (ALA), lignans, polyphenols, antioxidants, dietary fibre) are present in many plant sources and provide health benefits (anti-inflammatory, antimicrobial, antioxidant, hypoglycemic and hypolipidemic properties) beyond the primary nutritional value [1, 2] Linseed (*Linum usitatissimum* L.) has received much consideration and interest due to the beneficial fatty acid composition, mainly its richness in ALA precursor of the long-chain omega-3 polyunsaturated fatty acids (PUFA) [1, 3-5]. It has been reported that incorporation of different forms and varieties of linseed in human or animal diets influence the serum parameters, especially the lipid fraction [6-8]. Linseed addition has been found to be effective in quickly improve the ratio between n-6 and n-3 PUFA and raised the ALA content in pork meat [3, 9, 10].

Dietary modifying the pig meat lipids structure could help consumers meet health recommendations if we take into consideration the n-3 PUFA potential therapeutic roles [11].

The processing of walnut fruits (*Juglans regia* L.) results in considerable amounts of by-products including oilseed meals, with a higher content of omega-6 and omega-3 PUFA, which led to a beneficial effect on animal health [12].

* Corresponding author: Anca Gheorghe
Email: anca.gheorghe@ibna.ro

Moreover, the re-use of by-products as alternative sources of feed in farm animal diets beyond the nutritional aspects provide environmental and economic benefits [13].

To date, there is little information about the effects of walnut by-product in pigs' diets [2, 14] or their mixture with linseed [11, 15].

Therefore, the study aimed to assess the complementary effects of dietary n-3 PUFA rich mixture on growth performance, plasma metabolites and muscle fatty acids composition in growing pigs.

2. Materials and methods

Animals and experimental diets

All trial procedures were approved by the Animal Care Committee of the INCDBNA Balotesti (Romania), and pigs were handled in conformity with EU Directive 2010/63/EU [16].

Forty growing pigs Topigs [♀ Large White x Hybride (Large White x Pietrain) × ♂ Talent, mainly Duroc], average body weight 13.1±1.8 kg, were randomly allotted into two groups (male: female, 1:1) with 2 replicate each (10 pigs/pen).

The feed was given in the pelletized form. Free access to feed and water was provided for pigs all period. For 30 days feeding trial, pigs receive a control [C, based on corn-triticale-soybean meal (SBM)] or n-3 PUFA mix diet [extruded linseed EL: walnut meal WM, where 80 g/kg EL: WM mixture (50: 30 *wt/wt*) partially replace SBM]. The diets were isocaloric, isonitrogenous and meet nutrient requirements of pig's hybrid.

The analyzed composition of diets and dietary n-3 PUFA mix is given in Table 1. Dietary mixture addition higher the ALA concentration (5-fold) and lower the LA: ALA ratio (6-fold) of the experimental diet.

Table 1. Chemical composition of growing pig diets and dietary n-3 PUFA mixture

Item %	Diets		n-3 PUFA mixture (50: 30)
	C	EL: WM	
Dry matter	88.43	88.18	91.02
Crude protein	17.10	17.20	25.11
Lysine	1.05	1.05	0.91
Methionine + Cystine	0.67	0.67	0.98
Calcium	0.90	0.90	0.27
Phosphorus	0.70	0.70	0.84
Crude fibre'	4.66	4.97	15.93
Crude fat	4.37	4.80	16.02
ME (MJ/kg)[1]	12.67	12.66	15.36
Fatty acid (% of total FAME[2])			
Linoleic (LA)	50.14	40.10	35.67
α-linolenic (ALA)	4.48	22.46	35.91
LA: ALA ratio	11.19	1.79	1.00

C–control diet, EL: WM–extruded linseed:walnut meal diet. Results are given as mean (n=2)
[1]ME calculated using regression equations [17]; [2]FAME: fatty acids methyl esters

Pigs were weighed at the beginning, and the end of the feeding trial, feed intake (FI) was registered daily, to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain: feed ratio.

Sampling

Blood samples (n=8/group) were collected by jugular venipuncture in heparinized vacutainer tubes (6 mL) and centrifuged (3000 rpm for 15 min.) for plasma separation.

Longissimus dorsi muscle (200 g) samples (n=2/group) were collected at the end of the trial. The samples were individually vacuum-packed and stored at−18°C until analyses.

Chemical and fatty acids analyses

The proximate chemical composition (dry matter, crude protein, ether extract, crude fibre, and crude ash) were analyzed in duplicate using standardized methods [18].

Fatty acids composition analyses were done by gas chromatography method (SR CEN ISO/TS 17764-2:2008) using Perkin Elmer 500 chromatograph, as previously described [19]. Muscle samples were analyzed in duplicate.

Biochemical analyses

The plasma lipid (total cholesterol, TC; HDL-cholesterol, HDL-C; triglycerides, TG), protein (total protein, T-Pro; total bilirubin, T-Bil; albumin, Alb; creatinine, Cre; uric acid, UA; plasma urea nitrogen, PUN), mineral (calcium, Ca; magnesium, Mg, inorganic phosphorus, IP) and enzyme (glutamyl oxaloacetic transaminase, GOT; glutamyl pyruvic transaminase, GPT; creatine phosphokinase, CPK; gamma-glutamyl transferase, GGT) profiles were determined by Spotchem EZ SP-4430 chemistry analyzer (Arkray, Japan).

Statistical analysis

Data were analyzed by using one-way ANOVA in the GLM procedure of SPSS software (IBM SPSS Statistics version 20.0). Results are given as means and standard error of the mean (SEM). Differences were considered significant at $P \leq 0.05$.

3. Results and discussion

Growth performance

No significant difference (P>0.05) was observed on performance of growing pigs fed dietary n-3 PUFA mix (Table 2), even a slightly improved on final BW (+6%; P>0.05) and ADG (+8%; P>0.05) was noticed. The ADFI was similar between treatments (P>0.05), and the feed efficiency of pigs fed dietary n-3 PUFA mix tend to be improved (1.15 times higher in EL: WM group than C group; P=0.058).

There are a few previous studies reported results of feeding weaned piglets with different n-3 PUFA rich mixture, e. g. peas: linseed (3:1) with no influence on productive performance [1] or extruded linseed: walnut meal (8:1) mixture with a positive effect on body weight and average daily gain [15]. Recently, Hăbeanu et al., [11] stated that feeding barrows with 60 g/kg extruded linseed: walnut meal mixture (50:50 wt/wt) had no significant effect on growth performance but higher average daily gain (3.63%) and decrease the gain: feed ratio (5.22%) vs control diet. Tarricone et al., [20] also shown that feeding 3% extruded linseed in growing-fattening local swine breed for a long time (32 weeks) did not affect the growth performance (average daily gain, feed intake and feed conversion ratio).

Table 2. Effect of dietary n-3 PUFA mix on the performance of growing pig

Item	Diets		SEM	P-value*
	C	EL: WM		
No. pigs	20	20		
Body weight, Kg				
Initial (51 d)	13.10	13.50	0.32	0.140
Final (81 d)	30.12	31.93	0.62	0.166
ADG, kg/d	0.567	0.614	0.13	0.543
ADFI, kg/d	1.53	1.40	0.06	0.313
Gain : Feed ratio, kg: kg	0.38	0.44	0.10	0.058^{T}

C–control diet, EL: WM–extruded linseed: walnut meal diet; SEM, standard error of the mean. ADG, average daily gain; ADFI, average daily feed intake.*Means within rows do not differ significantly (P>0.05); T=Tendency to be influenced by treatment

Plasma metabolites

The effect of dietary n-3 PUFA mix on plasma metabolites response is presented in Table 3. Generally, at the end of the feeding trial, the plasma biochemistry parameters of both pigs' groups range in normal limits [21-24].

The results of our study showed that plasma lipid profile was affected as a response to n-3 PUFA rich mixture by decreasing the concentration of TC (7.84%; P=0.040), while the HDL-C, VLDL-C and TG concentrations insignificantly decreased compared with the control diet. Other study reported no significant effect on the plasma concentrations of glucose, cholesterol and triglycerides in the piglets fed with 3% nor 5% residual walnuts diets [2]. Gheorghe et al., [25] feeding piglets with 9% dietary mixture extruded linseed: walnut meal (8:1) reported no evident effect on plasma lipid profile (TC, HDL-C, and TG), and a positive correlation between dietary ALA fatty acids content and plasma HDL-C concentration. Our present results showed that the

dietary mixture had no effect on plasma T-Pro and its major fractions (Alb, UA), that are related to protein synthesis [26]. The dietary n-3 PUFA lowered PUN value (11.36%; P=0.045) that indicated a higher utilization of dietary nitrogen [27] and explained the growth performance of pigs; even the difference was not significant. Our study results showed that PUN: Cre ratio, an important indicator of renal disorders [23], range in normal limits (>10 mg/dL). The present results are in line with our previous study in weaned piglets that showed positive effects on growth performance and plasma protein profile, especially on PUN by feeding dietary extruded linseed: walnut meal (8:1) mixture [15]. Other study found that feeding weaned piglets with a pea: linseed mixture (3:1) are a good opportunity to optimize diet, with no significant effects on growth performance, lipid and protein biochemical profile, and urea: creatinine ratio [1]. Feeding pigs with n-3 PUFA-enriched diet did not significantly affect the plasma mineral profile and enzyme activity (P>0.05).

Table 3. Effect of dietary n-3 PUFA mix on plasma metabolites response

Plasma parameters	Limits	C	EL: WM	SEM	P-value
TC (mg/dL)	67.0-377[2]	112.85[a]	104.00[b]	2.28	0.040
HDL-C (mg/dL)	38.7-48.3[4]	47.71	45.14	1.52	0.471
VLDL-C (mg/dL)*	-	7.69	7.11	1.81	0.503
TG (mg/dL)	33-50[1]	38.43	35.57	0.36	0.503
T-Pro (g/dL)	5.0-8.3[1]	5.20	5.48	0.16	0.451
Alb (mg/dL)	2.3-4.0[1]	3.00	3.37	0.11	0.135
T-Bil (mg/dL)	0-0.5[1]	0.40	0.45	0.01	0.110
Cre (mg/dL)	0.8-2.3[1]	1.32	1.26	0.04	0.408
UA (mg/dL)	-	0.59	0.57	0.01	0.552
PUN (mg/dL)	8.2-25[1]	15.14[a]	13.42[b]	0.40	0.042
PUN: Cre ratio	10-20[3]	11.46	10.78	0.28	0.322
Ca (mg/dL)	6.8-14.8[2]	14.01	13.81	0.21	0.685
Mg (mg/dL)	2.3-3.5[1]	2.37	2.42	0.05	0.425
IP (mg/dL)	5.5-9.3[1]	8.98	8.77	0..13	0.485
GOT (U/L)	22-47[1]	23.43	22.14	2.18	0.737
GPT (U/L)	31-75[1]	52.00	53.71	2.29	0.715
CPK (U/L)	66-689[2]	344.00	284.00	45.98	0.188
GGT (U/L)	31-52[1]	43.85	43.57	4.30	0.977

C–control diet, EL: WM–extruded linseed: walnut meal diet; n=8 samples/group; SEM, standard error of means.
[ab]Means with different superscript within a row differ significantly (P<0.05).
[1]Merck Veterinary Manual, [21]; [2] Perri et al., [22]; [3]Washington and Van Hoosier, [23]; [4]Koreza et al., [24].
*VLDL-cholesterol=Triglycerides/5 [28].

Table 4. Effect of dietary n-3 PUFA mix on fatty acid composition of *Longissimus dorsi* muscle

Fatty acids, % of total FAME	C	EL: WM	SEM	P-value
C18:2n-6 (LA)	12.71	13.21	0.20	0.257
C18:3n-3 (ALA)	1.29[b]	1.46[a]	0.04	0.030
C20:5n-3 (EPA)	0.07	0.10	0.02	0.102
C22:5n-3 (DPA)	0.10	0.16	0.03	0.339
C22:6n-3 (DHA)	0.01[b]	0.05[a]	0.01	0.050
ΣSFA	38.41	38.88	0.16	0.151
ΣMUFA	42.53	41.46	0.20	0.104
ΣPUFA	16.69[b]	17.51[a]	0.23	0.000
Σn-6 PUFA	15.41	14.91	0.17	0.138
Σn-3 PUFA	1.93[b]	2.41[a]	0.12	0.019
n-6: n-3 ratio	7.98[a]	6.19[b]	0.41	0.002

C – control diet, EL: WM – extruded linseed: walnut meal diet. n=2 samples/group. SEM, standard error of means
[a,b]Means with different superscript within a row differ significantly (P<0.05).
ΣSFA=C14:0+C15:0+C16:0+C17:0+C18:0; ΣMUFA=C15:1+C16:1+C17:1+C18:1cis-9;
ΣPUFA=C18:2n-6+C18:3n-3+C20:2n-6+C20:3n-3+C20:4n-6+C20:5n-3.

Muscle fatty acid composition

The effect of dietary n-3 PUFA mix on the fatty acid composition of *Longissimus dorsi* muscle is shown in Table 4. As expected, the muscle of pigs fed dietary n-3 PUFA mix had higher contents in ALA (P=0.030), DHA (P=0.050), total PUFA (P=0.0001) and n-3 PUFA (P=0.019), and a lower n-6: n-3 ratio (P=0.002) compared with pigs fed a control diet with no adverse effect on the muscle quality parameters such as colour and texture (data not shown).

Similarly, Hăbeanu et al. [11] shown that the ALA concentrations increase in *Longissimus dorsi* and *Semitendinosus* muscles and n-6: n-3 ratio decreases as an effect of feeding barrows with 60 g/kg extruded linseed: walnut meal mixture (50:50 wt/wt).

Tarricone et al., [20] shown that feeding 3% extruded linseed in growing-fattening local swine breed improved the fatty acid profile of meat increasing the n-3 PUFA and decreasing the n-6:n-3 ratio, with benefits for human health. Nevrkla and Vaclavkova, [29] confirmed that feeding 7% linseed scrap in gilts diet positively influenced the fatty acid profile in the intramuscular fat and backfat with no adverse effect on the oxidative stability of the fat in the meat. Other research proved that utilisation of extruded or co-extruded linseed up to 5% improves the fatty acid profile of the pork meat [30-32].

4. Conclusions

The use of 80 g/kg dietary n-3 PUFA rich mixture based on extruded linseed: walnut meal (50:30 wt/wt) had a positive effect on growing pigs' performance and health status improving the plasma lipo-protein response. Dietary n-3 PUFA rich mixture enhanced the n-3 fatty acid profile of the meat.

Acknowledgements

This work was funded by the Romanian Ministry of Education and Research through Subprogram 1.2. Institutional Performance, Program 1. Developing National R&D, National Research and Development and Innovation Contract no. 17PFE/2018, Projects PN18-20.01.03 and PN19-09.01.04.

References

1. Hăbeanu, M., Lefter, N., Gheorghe, A., Tabuc, C., Dumitru, M., Ciurescu, G., Palade, M., Effects of dietary peas mixed with linseed (3:1) on the growth performance, enteritis and certain serum parameter in weaned piglets, Food and Feed Research, 2017, 44(2), 173–180.

2. Taranu, I., Habeanu, M., Palade, L. M., Marin, D., Beneficial effect of dietary bioactive compounds from residual nuts, a by-product of pastry on antioxidant defense in pigs after weaning, Scientific Papers: Animal Science and Biotechnologies, 2018, 51(1), 50–54.

3. Quiniou, N., Goues, T., Vautier, A., Nassy, G., Chesneau, G., Weill, P., Etienne, M., Mourot, J., Consequence of extruded linseed incorporation in sows and/or pigs' diets on performance. Conference at the 61st Annual Meeting of the European Association for Animal Production, Heraklion, Crete Island, Greece, 2010, pp. 5.

4. Beckova, B., Vaclavkova, E., The effect of linseed diet on carcass value traits and fatty acid composition in muscle and fat tissue of fattening pigs, Czech Journal of Animal Science, 2010, 55, 313–320.

5. Jasinska, K., Kurek, M.A., The effect of oil plants supplementation in pig diet on quality and nutritive value of pork meat, Animal Science Papers and Reports, 2017, 35, 137–146.

6. Barceló-Coblijn, G., Murphy, E. J., Othman, R., Moghadasian, M. H., Kashour, T., Friel, J. K., Flaxseed oil and fish-oil capsule consumption alters human red blood cell n-3 fatty acid composition: a multiple-dosing trial comparing 2 sources of n-3 fatty acid, The American Journal of Clinical Nutrition, 2008, 88(3), 801-809.

7. Goyal, A., Sharma, V., Upadhyay, N., Gill, S., Sihag, M., Flax and flaxseed oil: an ancient medicine & modern functional food, 2014, The Journal of Food Science and Technology, 51(9), 1633–1653.

8. Taranu, I., Gras, M., Pistol, G. C., Motiu, M., Marin, D. E, Omega-3 PUFA rich camelina oil by-products improve the systemic metabolism and spleen cell functions in fattening pigs, PLoSOne, 2014, 9(10), e110186.

9. Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Effects of fatty acids on meat quality: a review, Meat Science, 2003, 66, 21–32.

10. Urrutia, O., Mendizabal, J. A., Insausti, K., Soret, B., Purroy, A., Arana, A., Effects of addition of linseed and marine algae to the diet on adipose tissue development, fatty acid profile, lipogenic gene expression, and meat quality in lambs, PLoSOne, 2016, 11(6), e0156765.

11. Hăbeanu, M., Lefter, N. L., Gheorghe, A., Untea, A., Ropotă, M., Grigore, D. M., Vărzaru, I., Toma, S. M., Evaluation of performance, nitrogen metabolism

and tissue composition in barrows fed an n-3 PUFA-rich diet, Animals, 2019, 9, 234.
12. Jahanban-Esfahlan, A., Ostadrahimi, A., Tabibiazar M., Amarowicz, R., A comparative review on the extraction, antioxidant content and antioxidant potential of different parts of walnut (*Juglans regia* L.) Fruit and tree, Molecules, 2019, 24(11), 2133.
13. Fernández-Agulló, A., Pereira, E., Freire, M. S., Valentão, P., Andrade, P. B., González-Álvarez, J., Pereira J. A., Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts, Industrial Crops and Products, 42, 126-132.
14. Habeanu, M., Voicu, D., Lefter, N., Gheorghe, A., Ropota, M., Vlad, C., Ghita, E., Effects of the full fat walnuts waste on the fatty acids composition of the hepatic and cardiac tissue in Topigs piglets, 66th Annual Meeting of the European Association for Animal Production, Warsaw, Poland, 2015, pp. 267.
15. Gheorghe, A., Hăbeanu, M., Lefter, N. L., Grigore, D. M., Effect of dietary extruded linseed and walnut meal mixture (8:1) on performance and plasma protein profile in weaned piglets, Bulletin UASVM Animal Science and Biotechnologies, 2018, 75(2), 121–126.
16.Official Journal of the European Union, Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes, 2010, Series L 276, 33-79.
17. Nutrient Requirements of Swine, Revised 10th ed., National Academy Press, Washington, DC, 1998.
18. Official Journal of the European Union L 54, Commission Regulation (EC) No. 152/2009 laying down the methods of sampling and analysis for the official control of feed, 2009, Series L 155:1–66.
19. Hăbeanu, M., Lefter, N., Gheorghe, A., Al. Nagy, Marin, D., Ropotă, M., Effects of dietary flaxseed oil on the muscle fatty acid composition in Mangalitsa pigs in an extensive rearing system, South African Journal of Animal Science, 2014, 44(3), 240–244.
20. Tarricone, S., Colonna, M. A., Giannico, F., Ragni, M., Lestingi, A., Facciolongo, A. M., Effect of an extruded linseed diet on meat quality traits in Nero Lucano pigs, South African Journal of Animal Science 2019, 49 (6), 1093-1103.
21. Merck&Co., The Merck Veterinary Manual, 10th ed., Merck Co., Inc., Kenilworth, NJ, USA, 2010.
22. Perri, A. M., O'Sullivan, T. L., Harding, J. C. S, Wood, R. D., Friendship, R. M., Haematology and biochemistry reference intervals for Ontario commercial nursing pigs close to the time of weaning. Canadian Veterinary Journal, 2017, 58(4), 371–376.
23. Washington, I. M., Van Hoosier, G., The laboratory rabbit, guinea pig, hamster, and other rodents, Clinical Biochemistry and Hematology, 2012, doi: 10.1016/B978-0-12-380920-9.00003-1.
24. Kozera, W. J., Karpiesiuk, K. D., Bugnacka, J. Falkowski, Milewska, W, Production performance of pigs reared in different systems and fed increased energy content diets with or without green alfalfa, South African Journal of Animal Science, 2016, 46 (1), 70-76.
25. Gheorghe, A., Hăbeanu, M., Lefter, N. A., Dumitru, M., Grigore, D. M., Aspects of plasma biochemistry and intestinal health of weaned piglets fed dietary extruded linseed and walnut meal mixture, Food and Feed Research, 2019, 46(1), 147-153.
26. Hellwing, A., Louise, F., Tauson, A.H., Skrede, A., Blood parameters in growing pigs fed increasing levels of bacterial protein meal. Brief communication, Acta Veterinaria Scandinavica, 2007, 49(33), 1-4.
27.Shen, Y. B., Voilque, G., Kim, J. D., Odel, J., Kim, S. W., Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs, Journal of Animal Science, 2012, 90, 2264-2275.
28. Tietz, N. W., Fundamentals of clinical chemistry. 4th ed. Philadelphia: W.B. Saunders Company, 1996.
29. Nevrkla, P., Vaclavkova, E., The effect of diet supplementation with linseed scrap on the meat quality and fatty acid profile of the meat and backfat in fattening gilts, Veterinarni Medicina, 2019, 64, 467–475.
30. Corino, C., Musella, M. Mourot, J., Influence of extruded linseed on growth, carcass composition and meat quality of slaughtered pigs at one hundred and one hundred sixty kilograms of liveweight, Journal of Animal Science, 2008, 86, 1850–1860.
31. Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T., Beaulieu, A. D., Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability, Food Chemistry, 2011, 126, 1716–1723.
32. De Tonnac, A., Karim-Luisset, S., Mourot, J., Effect of different dietary linseed sources on fatty acid composition in pig tissues, Livestock Science, 2017, 203, 124–131.