The effects of millet and a mix of extruded linseed: walnut meal on certain plasma parameters in growing-fattening pigs

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ABSTRACT

The purpose of this experimental study was to highlight the effects of millet either separate or associated with a mix of extruded linseed: walnut meal, 50:50 (wt:wt), on plasma parameters in growing-fattening pigs. The experiment was carried out for 3 weeks on 15 Topigs hybrids, 81 ± 3 days old, with an initial body weight of 31.5 Kg, distributed into 3 groups (5 replicates / group): negative control (M-, corn, triticale, soybean meal); experimental 1 (E1, corn, millet, soybean meal); experimental 2 (E2, millet mixed with 1:1 extruded linseed: walnut meal). At the beginning and the end of the experiment, all pigs were used for blood sample collections from the jugular vein to determine the biochemical profiles using the Spotchem EZ SP-4430 analyzer (Arkray, Japan). The statistically significant differences between groups were found for two plasma parameters, respectively total protein (<1.14 times in E2 group compared to E1 group), uric acid (<1.11 times in E2 group compared to E1 group). The results of our analysis ranged according to the reference interval, suggesting a good state of health.

Keywords: growing-fattening pigs, millet, extruded linseed: walnut meal mix, plasma profile

INTRODUCTION

The pigs organism's response to the quality and composition of a certain type of feed may give us information about health status reflected by the concentration of certain biochemical markers (Habeanu et al., 2015). A good health state would be in a positive connection with the pigs' development. Diversifying the structure of mixed feed recipes appears as a necessity dictated by the need of using either the locally available resources or the cheaper ones. Millet is an important energy source, a potential substitute for corn, triticale or other vegetable resources which covers a high proportion of essential amino acids and oils required by young swine. A millet-based diet given to pigs may improve both the feed intake and the weight gain when compared to a conventional diet (Yin et al., 2002). In previous studies, there is a very small amount of scientific data regarding the effects of the addition of millet grains in the pigs' diet; however, this feed ingredient was found beneficial for the broiler chickens' growth performances (Torres et al., 2013).

The studies performed over the last decades have highlighted the close correlation between certain bioactive principles in the feed ingredients and the healthy state of animals. Thus, a series of resources are known as valuable polyunsaturated fatty acids (PUFA) sources, especially α-linolenic acid (Ciucă et al., 2013, Hăbeanu et al., 2014[a]). Establishing values close to 1 for the ratio of n-6 : n-3 PUFAs is a very important condition for a good state of health (Li et al., 2015[a]).

Swine use well the highly digestible flaxseeds (Ndou et al., 2017, Habeanu et al., 2019). The content of omega-3 fatty acids is particularly beneficial to the animal organism, having positive effects both on the quality of products and on the specific health markers (Juárez et al., 2010).

Even though the walnut by-products exert a favorable influence, their use in animal feeding is not a topic commonly found in scientific papers or practice. The studies approaching the nutritional properties of walnuts are mainly medical and refer especially to the laboratory rats as experimental animals (Alsuhaibani & Al-Kuraieef, 2019). These by-products rich in proteins, minerals, and PUFA are a potential alternative to the conventional cereal – soybean mixtures suitable for young swine (Hăbeanu et al., 2014[b]; Gheorghe et al., 2018). Research on other monogastric such as the Japanese quail showed appropriate metabolic and productive responses of the organism during the growth period (Arjomandi & Salarmoini, 2016). Gheise et al., (2018) treated in their research the influence of walnut by-products on metabolism in dairy cows.

The plasma biochemical components serve as specific markers of the animals' state of health (Kumar et al., 2017, Habeanu et al., 2011) being also important in the nutritional assessment (Bakare et al., 2016). The studies in the physiological field would typically determine blood urea nitrogen (BUN), total protein (T-Pro) (Li et al., 2015[b]), total bilirubin (T-Bil), gamma-glutamyl transferase (GGT) (Abeni et al., 2018), uric acid (UA) and creatine phosphokinase (CPK) (Bakare et al., 2016) concentrations. There seems to be a connection between dietary protein intake and the BUN concentration (Fang et al., 2018). The inappropriate concentrations of BUN not matching with the reference interval values are associated with kidney disease (Upadhaya et al., 2016). A similar mismatch in the case of T-Bil and GGT means susceptibility of liver disease, and a lack of correspondence for the lactate dehydrogenase (LDH) activity might indicate muscle tissue damage (Abeni et al., 2018). In their studies, Sutherland et al. (2012) mentioned an increasing activity of the CPK after the organism's exposure to stressful conditions. Bakare et al., (2016) studied the effect of the fiber inclusion in the finishing pigs' diet on the CPK activity without finding any changes related to nutritional stress. Studies in the pig's hematological profile are quite rare in recent years.

This paper aimed to evaluate the influence of millet single or associated with a mixture between 2 omega-3 rich sources (extruded linseed: walnut meal) on the plasma biochemical profile of the growing - fattening pig, which gives some indication about animal's state of health.

MATERIAL AND METHODS

The experimental procedures were approved by the Ethics Commission of the National Research-Development Institute for Animal Biology and Nutrition, Balotesti, Romania and followed the EU Directive 2010/63/EU (OJEU, 2010).

Animals and diets

The biological testing was carried out on 15 growing-fattening pigs, Topigs hybrid (♀ Large White × Large White × Pietrain × ♂ Talent, mainly Duroc), 81 ± 3 days, with an initial average weight of 31.5 Kg, housed in individual metabolic cages for 21 days. The pigs were randomly assigned to 3 groups (n = 5): negative M (mixed feed based on corn, 25% triticale and soybean meal), an experimental group E1 (corn, soybean meal and 25% millet) and the experimental group E2 fed a diet containing corn, 25% millet associated with a mixture of extruded linseed and walnut meal at a 1:1 ratio. The recipes were formulated isoenergetic and isonitrogenous according to the specific nutritional requirements of the TOPIGS hybrid, with similar content of essential amino acids, Ca and P (Habeanu et al., 2019). The experimental animals were offered *ad libitum* access to feed and water.

Measurements and analyses

At the beginning and the end of the experiment blood samples were collected by venipuncture, from the jugular veins, under aseptic conditions, from all animals (n = 15). The samples were prepared by centrifugation to extract the plasma for the determination of the biochemical parameters. The analysis of plasma samples assumed the following profiles: proteic (T-

Pro, total protein; T-Bil, total bilirubin; Alb, albumin; Cre, creatinine; BUN, blood urea nitrogen; UA, uric acid), energetic (T-Chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides), enzymatic [GGT, gamma-glutamyltransferase; GOT (AST) glutamic-oxaloacetate transaminase (aspartate aminotransferase); GPT (ALT), glutamyl-pyruvate transaminase (alanine aminotransferase); LDH, lactate dehydrogenase] and mineral (Ca, calcium; Mg, magnesium; P, phosphorus) which were determined using the Spotchem EZ SP-4430 analyzer, Arkray, Japan.

Statistical analyses

The plasma parameter values were introduced in IBM SPSS Statistics software version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0) for statistical data analysis. We used the Tukey test to see the differences within the groups, which involved the multiple comparisons of means. Data concerning the diet influence on the biochemical plasma profile were presented as mean values and the standard error of the mean.

RESULTS AND DISCUSSION

The studies focused on the assessment of the health status and the nutritional condition based on a particular type of feed relies on the blood plasma parameters (Hăbeanu et al., 2011, (Li et al., 2015[b]). The mean concentrations of the protein (T-Pro, T-Bil, Alb, Cre, BUN, and UA), energetic (T-Chol, HDL-C, TG), enzymatic (GGT, AST, ALT, LDH) and mineral components (Ca, Mg, IP) are presented into the Tables 1-4.

The protein profile

The studies on plasma metabolites usually provide information about the animals' health status which is linked both to the absorption of the optimum nutrients and to the growing performances. Table 1 shows the plasma protein concentration and of their fraction (T-Bil, Alb, Cre, BUN and UA). In our study, the plasma protein parameters that changed significantly ($P < 0.05$) as a result of the composition of the diet given to pigs were T-Pro and UA. A mean value of the T-Pro slightly below the normal reference range (The Merck Veterinary Manual, 2010) might denote a deficiency of the metabolism of this category of nutrients. This was probably the case recorded in the E2 group where T-Pro reached 5.22 g/dL. For the rest of the groups, the T-Pro ranged within the physiologically normal limits (5.8 - 8.3 g / dL, The Merck Veterinary Manual, 2010). In the case of the E1 diet, the mean values of T-Pro increased by 0.67% compared to those corresponding to the M diet. In the other experimental treatment, E2, a decrease of 13.79% in T-Pro level was found in relation to M. The changes in this parameter denote an optimal use of the E1 diet and an appropriate physiological condition. In the young swine, the nutritional adequacy of the diets to the growing organism requirements influences the level of T-Pro (Liu et al., 2015). Hăbeanu et al. (2017) providing a mix of peas and linseed as an experimental diet for this category of swine did not find statistically significant differences between groups. While making investigations on the role of soy oil in pigs' nutrition, Lv et al. (2018) noticed the fact that, in a first phase, the T-Pro decreased nearly significantly, and later, by the end of the experiment, this parameter did not record statistically significant changes anymore.

As part of the metabolic wastes, the UA denotes a good health status of the animal and the positive effects of the feed on the organism as long as its level is low. Among other metabolic components, the UA makes a useful biomarker for establishing the diagnostic of nutritional health in pigs. It was included in the experimental research carried out by Bakare et al. (2016). According to the statistical results obtained by these authors, this analyze presented, however, no significant changes between the groups of experimental animals. Czech et al. (2018) noticed statistically significant changes in the UA level corresponding to the addition of probiotics and yeasts in the pigs' diets. In our case, the mean level of UA was also significantly affected by the diet content. In the E1 treated group, this component decreased by 3.33% and in E2 it dropped by 14.81% compared with the M treated group.

By millet dietary addition single or in association with 1:1 extruded linseed and walnut meal, T-Bil did not alter statistically significant between the experimental groups of animals and the M group. According to the results obtained by Abeni et al., (2018), the levels of this parameter increased significantly as a result of feeding the pigs a diet without soybean meal supplemented with crystalline amino acids. In the research protocol of these authors, the T-Bil was considered one of the markers for the assessment of the hepatobiliary injury.

After introducing lysine in the pigs' diet, Regmi et al., (2017) noticed an increased concentration of Alb in the blood plasma level. Liu et al., (2015) found a statistically significant increase in the plasma Alb levels in pigs whose diet had been experimentally formulated with a reduced protein/energy ratio, compared to the animals fed with a conventional diet. Huang et al., (2019) analyzed the Alb and other serum analyzed concentrations in the pigs given lipidic supplements in their diet to find a significant increase in their level compared to the control animals, which had not been offered such ingredients in their diet. Contrary, at the end of our experimental study, the Alb decrease in the E2 group by 3.42% compared to the M group but not record significant statistical differences.

In E1 group our data were in accord with the found of the above-mentioned authors (5.52% higher than the M group).

While analyzing the serum parameters Cre and BUN obtained from pigs after certain treatments with probiotics and exogenous enzymes (protease), Payling et al., (2017) recorded a statistically significant increase in the concentration of Cre and reduction in the level of the BUN respectively. Zielonka et al., (2010) investigated the physiological condition in boars after two nutritional treatments based on proteases and peptidases. The blood samples collected from those animals provided statistically significant reductions in the levels of serum Cre associated with the proportions of the exogenous enzymes included in the diet. At the end of the nutritional tests initiated by Lv et al., (2018), the serum concentration of BUN increased significantly as a result of glutamine use. In our nutritional experiment, there were noticed lower concentrations of Cre but these changes were not statistically significant ($P > 0.05$). When there is an optimum level of dietary-protein available, BUN tends to decrease (Abeni et al., 2018; Liu et al., 2015) and so do other metabolites such as the amino acids and assimilated nitrogen (Xie et al., 2013). Gheorghe et al., (2018) found significant decreased mean values for BUN following an experimental diet of extruded linseed and walnut meal. In our experiment, mean BUN concentrations did not indicate significant differences between groups ($P = 0.16$). Exceeding the reference interval for this plasma parameter may be associated with renal disorders (Upadhaya et al., 2016).

Table 1. Plasma proteic profile

Plasma parameters	Reference values		Mean values			SEM[7]	*P-value*
			M	E1	E2		
T-Pro[1] (g/dL)	4.9 - 6.7*	5.8 - 8.3**	5.94[ab]	5.98[a]	5.22[b]	0.15	*0.04*
T-Bil[2](mg/dL)		0 - 0.5**	0.20	0.24	0.20	0.01	*0.11*
Alb[3] (g/dL)	1.9 - 2.9*	2.3 - 4.0**	3.62	3.82	3.50	0.08	*0.27*
Cre[4] (mg/dL)	0.99 - 1.47*	0.8 - 2.3**	1.34	1.32	1.26	0.02	*0.35*
BUN[5] (mg/dL)	4.77 - 12.62*	8.2 - 25**	9.80	11.80	12.90	0.67	*0.16*
UA[6] (mg/dL)	–	–	0.62[a]	0.60[ab]	0.54[b]	0.01	*0.02*

Note: *Klem et al. (2010);**Merck Veterinary Manual (2010). Tenth Edition. Merck &Co. Inc., USA; [1]T-Pro, total protein; [2]T-Bil, total bilirubin; [3]Alb, albumin; [4]Cre, creatinine; [5]BUN, blood urea nitrogen; [6]UA, uric acid; [7]SEM, standard error of the mean. Different letters between columns denote significant differences ($P < 0.05$)

The energetic profile

For the energetic profile shown in Table 2, no significant changes were found between the groups as a response to the diet types fed to the animals in our experiment. There was noticed, however, a tendency in the applied treatments to affect (P = 0.08) in the case of TG. Liu & Kim (2018) noticed a significant reduction in the concentrations of TG that was also recorded in the case of T-Chol and HDL-C as a result of the PUFA (n-6:n-3) intake. This reduction appeared to be generated by the 5:1 ratio of the linoleic and α-linolenic fatty acids.

By our experiment, we noticed a decrease in the concentration of HDL-C which was statistically insignificant (<11.22% in the E2 group compared to the M group). In their studies concerning the changes induced by the probiotics in the serum parameters of the swine, Joysowal et al., (2018) noticed significant reductions in the concentrations of TG and T-Chol as a result of providing host-specific strains compared to the control treatment and the treatment with probiotics strains not specific to the pig.

Table 2. Plasma energetic profile

Plasma parameters (mg/dL)	**Reference values**		**Mean values**			**SEM**	***P-value***
			M	E1	E2		
T-Chol[1]	77.2 -162.16*	81- 134**	113.20	101.20	107.80	2.674	*0.19*
HDL-C[2]	–	–	43.60	42.40	39.20	1.680	*0.58*
TG[3]	20 - 238*	–	27.60	19.40	32.20	2.408	*0.08*

Note: *Klem et al. (2010);**Merck Veterinary Manual (2010). Tenth Edition. Merck &Co. Inc., USA; [1]T-Chol, total cholesterol; [2]HDL-C, high-density-lipoprotein cholesterol; [3]TG, triglyceride.

The enzymatic profile

Regarding the enzymatic activity (GGT, GOT, GPT and LDH, table 3) changed not significantly (P > 0.05) when adding millet to the diet, either separately or associated with extruded linseed: walnut meal.

Abeni et al. (2018) studied GGT activity as a marker of health in pigs without finding significant differences between the experimental groups fed on a low-protein diet supplemented with crystalline amino acids and the control group. According to the nutritional studies carried out by Bakare et al., (2016), a diet with a high content of fiber causes an increase in CPK activity, in pigs.

On the contrary, Zielonka et al. (2010) noticed statistically increased levels of Ca, Mg and IP but the effect was due to the exogenous enzymes administrated to the boars. The experimental groups recorded highly significant differences for the mean concentrations of Mg and significant differences for Ca depending on the dose of the corresponding treatment. The IP also recorded significant changes in relation to the introduction of enzymatic feed additives in the diet. Samolińska et al., (2019) carried out research experiments on the dietary inulin inclusion to see its influence on some plasma mineral parameters including Mg in pigs without recording any significant differences between the treatments applied to the animals.

Table 3. Plasma enzymatic profile

Plasma parameters (IU/L)	Reference values		Mean values			SEM	P-value
			M	E1	E2		
GGT[1]	0 - 82*	–	62.33	63.80	52.00	3.70	*0.40*
GOT (AST)[2]	0 - 125*	15 - 55**	27.13	58.70	60.40	6.67	*0.11*
GPT (ALT)[3]	0 - 103*	22 - 47**	41.00	46.10	41.90	2.05	*0.72*
LDH[4]	0 - 1893*	160- 425**	1198.40	1550.00	1405.60	91.19	*0.50*

Note: *Klem et al. (2010);**Merck Veterinary Manual (2010). Tenth Edition. Merck &Co. Inc., USA; [1]GGT, gamma-glutamyltransferase; [2]GOT, glutamic-oxaloacetat transaminase; [3]GPT, glutamic-pyruvic transaminase; [4]LDH, lactate dehydrogenase; IU, international units per litre.

The mineral profile

According to our experimental results, the dietary treatments did not produce significant changes in the mean values of the minerals concentrations relative to the M group (Table 4).

The blood metabolites level records certain diet-dependent variations. Soybean, for example, is among the most suitable products for growing pigs due to the considerable N, Ca and P intake compared to other protein-rich feed ingredients (Kumar et al., 2017).

Concerning the metabolic need of P, Hăbeanu et al. (2011) mentioned in their studies that a reduction in the blood glucose level may also affect the blood P concentration. In a phytase treatment provided to pigs, Cowieson et al., (2017) noticed that there were significantly increased concentrations of P and insignificant changes in the Ca level in plasma. According to our results, the mean plasma concentration values of P did not indicate significant differences between the pig's groups ($P > 0.05$) and Mg level neither.

Table 4. Plasma mineral profile

Plasma parameters (mg/dL)	Reference values		Mean values			SEM	*P-value*
			M	E1	E2		
Ca[1]	10 - 12.4*	9.3 -11.5**	14.74	14.76	13.80	0.30	*0.36*
Mg[2]	2.19 - 2.92*	2.3 - 3.5**	1.90	2.08	1.90	0.05	*0.21*
IP[3]	8.66 - 13.31*	–	8.46	8.20	7.58	0.20	*0.20*

Note: *Klem et al. (2010);**Merck Veterinary Manual (2010). Tenth Edition. Merck &Co. Inc., USA; [1]Ca, calcium; [2]Mg, magnesium; [3]IP, inorganic phosphorus.

CONCLUSIONS

Two of the investigated plasma parameters, T-Pro and UA provided supportive evidence in assessing the influence of the nutritional factor on the physiological condition of the pigs. The significant differences we found between groups for these biochemical parameters might be related both to the concentration of the unconventional ingredients in the feed structure and to the PUFA content of the diet.

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REFERENCES

Abeni, F., Petrera, F., Dal Prà, A., Rapetti, L., Crovetto, G. M., & Galassi, G. (2018). Blood parameters in fattening pigs from two genetic types fed diet with three different protein concentrations. Translational Animal Science, 2(4), 372-382.

Alsuhaibani, A. M., & Al-Kuraieef, A. N. (2019). Effect of phenolic compounds and fatty acid contents of walnut seeds on streptozotocin-induced diabetes in rats. Journal of Food Measurement and Characterization, 13(1), 499-505.

Arjomandi, M. A., & Salarmoini, M. (2016). Walnut Meal as an Excellent Source of Energy and Protein for Growing Japanese Quails. Iranian Journal of Applied Animal Science, 6(4), 925-930.

Bakare, A. G., Ndou, S. P., Madzimure, J., & Chimonyo, M. (2016). Nutritionally related blood metabolites and performance of fattening pigs fed on graded levels of dietary fibre. Tropical animal health and production, 48(5), 1065-1069.

Ciucă, N., Drăgotoiu, D., Gheorghe, A., Ciurescu, G., & Hăbeanu, M. (2013). Effect of the dietary by-product camelina meal on performances and carcass quality of TOPIGS pigs. Bulletin UASVM Animal Science and Biotechnologies, 70(2): 205-213.

Cowieson, A.J., Roos, F.F., Ruckebusch, J.P., Wilson, J.W., Guggenbuhl, P., Lu, H., Ajuwon, K.M. and Adeola, O. (2017). Time-series responses of swine plasma metabolites to ingestion of diets containing myo-inositol or phytase. British Journal of Nutrition, 118(11), pp.897-905.

Czech A, Smolczyk A, Ognik K, Wlazło Ł, Nowakowicz-Dębek B, Kiesz M (2018) Effect of dietary supplementation with *Yarrowia lipolytica* or *Saccharomyces cerevisiae* yeast and probiotic additives on haematological parameters and the gut microbiota in piglets. Research of Veterinary Science, 119:221–227.

Fang, L. H., Jin, Y. H., Do, S. H., Hong, J. S., Kim, B. O., Han, T. H., & Kim, Y. Y. (2018). Effects of dietary energy and crude protein levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs. Asian-Australasian Journal of animal Sciences 32(4), 556-563.

Gheise, N. J. E., Riasi, A., Celi, P., & Shahneh, A. Z. (2018). Effects of dietary supplementation of pioglitazone or walnut meal on metabolic profiles and oxidative status in dairy cows with high pre-calving BCS. Journal of Dairy Research, 85(1), 16-22.

Gheorghe, A., Hăbeanu, M., Lefter, N. A. & Grigore, D. M. (2018). 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, 75(2), 121-126.

Hăbeanu M., Lefter N.A., Gheorghe A., Tabuc C., Untea A., Surdu I., Ciurescu G., Balan C.G.& Dragomir C. (2015).Changes in certain serum and faeces parameters in weaned piglets as a response to nutritional stress. South African Journal of Animal Science, 45 (No. 2).

Hăbeanu, M., Lefter, N., Gheorghe, A., Nagy, A. I., Marin, D., & Ropota, M. (2014[a]). 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, 44(3), 240-244.

Hăbeanu, M., Gheorghe, A., Lefter, N., Ropotă, M. (2014[b]). Subproduse de nucă – ingrediente furajere cu potenţial nutriţional valoros în hrana tineretului porcin, Analele INCDBNA vol. 30: 13-22

Hăbeanu, M., Hebean, V., Țăranu, I., Ropota, M., Lefter, N. & Marin, D. (2011). Dietary ecologic camelina oil-a beneficial source of n-3 PUFA in muscle tissue and health status in fattening pigs. Romanian Biotechnological Letter, 16, 5564-5571.

Hăbeanu, M., Lefter, N., George, A., Tabuc, C., Dumitru, M., Ciruescu, G., & Palade, M. (2017). 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, 44(2), 173-180.

Hăbeanu M., Lefter N.A., Gheorghe A., Untea A., Ropotă M., Grigore D.-M., Varzaru I. and Toma S.M. (2019). Evaluation of Performance, Nitrogen Metabolism and Tissue Composition in Barrows Fed an n-3 PUFA-Rich Diet. Animals. 9, 234.

Huang, C., Chiba, L. I., Magee, W. E., Wang, Y., Griffing, D. A., Torres, I. M., ... & Spangler, E. A. (2019). Effect of flaxseed oil, animal fat, and vitamin E supplementation on growth performance, serum metabolites, and carcass characteristics of finisher pigs, and physical characteristics of pork. Livestock Science, 220, 143-151.

Joysowal, M., Saikia, B. N., Dowarah, R., Tamuly, S., Kalita, D., & Choudhury, K. D. (2018). Effect of probiotic *Pediococcus acidilactici* FT28 on growth performance, nutrient digestibility, health status, meat quality, and intestinal morphology in growing pigs. Veterinary world, 11(12), 1669-1676.

Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T., & Beaulieu, A. D. (2010). Feeding co-extruded flaxseed to pigs: Effects of duration and feeding level on growth performance and backfat fatty acid composition of grower–finisher pigs. Meat Science, 84(3), 578-584.

Klem, T. B., Bleken, E., Morberg, H., Thoresen, S. I., & Framstad, T. (2010). Hematologic and biochemical reference intervals for Norwegian crossbreed grower pigs. Veterinary clinical pathology, 39(2), 221-226.

Kumar, H., Kumar, B., & Singh, S. K. (2017). Effect of replacing fish meal and ground nut cake by doc soya on weight gain, digestibility and blood biochemical profiles in growing pigs. Indian Journal of Animal Research, 51(4) pp.722-725.

Lefter, N. (2013). Cercetări privind utilizarea unor materii prime furajere non-convenţionale în hrana porcilor la îngrăşat (Teză de doctorat), Universitatea de Ştiinţe Agronomice şi Medicină Veterinară Bucureşti, Facultatea de Zootehnie.

Lawrence, B. V., Adeola, O., & Rogler, J. C. (1995). Nutrient digestibility and growth performance of pigs fed pearl millet as a replacement for corn. Journal of Animal Science, 73(7), 2026-2032.

Li, F., Duan, Y., Li, Y., Tang, Y., Geng, M., Oladele, O. A., & Yin, Y. (2015a). Effects of dietary n-6: n-3 PUFA ratio on fatty acid composition, free amino acid profile and gene expression of transporters in fattening pigs. British Journal of Nutrition, 113(5), 739-748.

Li, Y., Zhang, H., Yang, L., Zhang, L., & Wang, T. (2015b). Effect of medium-chain triglycerides on growth performance, nutrient digestibility, plasma metabolites and antioxidant capacity in weanling pigs. Animal Nutrition, 1(1), 12-18.

Liu, W. C. & Kim, I. H. (2018). Effects of different dietary n-6: n-3 PUFA ratios on growth performance, blood lipid profiles, fatty acid composition of pork, carcass traits and meat quality in finishing pigs. Annals of Animal Science, 18(1), 143-154.

Liu, Y., Kong, X., Jiang, G., Tan, B., Deng, J., Yang, X., Li, F., Xiong, X., Yin, Y., (2015). Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes. Journal of Animal Science and Biotechnolology 6, 36.

Lv, D., Xiong, X., Yang, H., Wang, M., He, Y., Liu, Y., & Yin, Y. (2018). Effect of dietary soy oil, glucose, and glutamine on growth performance, amino acid profile, blood profile, immunity, and antioxidant capacity in weaned piglets. Science China Life Sciences, 61(10), 1233-1242.

Manual, M.V., 2010. A handbook of diagnosis, therapy, and disease prevention and control for the veterinarian. Eds: Kahn, CM & Line, S., Merck and Co. Inc., New Jersey, USA, pp.905-908.

Ndou, S. P., Kiarie, E., Thandapilly, S. J., Walsh, M. C., Ames, N., & Nyachoti, C. M. (2017). Flaxseed meal and oat hulls supplementation modulates growth performance, blood lipids, intestinal fermentation, bile acids, and neutral sterols in growing pigs fed corn–soybean meal–based diets. Journal of Animal Science, 95(7), 3068-3078.

Payling, L., Kim, I. H., Walsh, M. C., & Kiarie, E. (2017). Effects of a multi-strain Bacillus spp. direct-fed microbial and a protease enzyme on growth performance, nutrient digestibility, blood characteristics, fecal microbiota, and noxious gas emissions of grower pigs fed corn-soybean-meal-based diets—A meta-analysis. Journal of Animal Science, 95(9), 4018-4029.

Regmi, N., Wang, T., Crenshaw, M. A., Rude, B. J., & Liao, S. F. (2018). Effects of dietary lysine levels on the concentrations of selected nutrient metabolites in blood plasma of late-stage finishing pigs. Journal of Animal Physiology and Animal Nutrition, 102(2), 403-409. doi:10.1111/jpn.12714.

Samolińska, W., Grela, E. R., & Kiczorowska, B. (2019). Effects of inulin extracts and inulin-containing plants on haematobiochemical responses, plasma mineral concentrations, and carcass traits in growing-finishing pigs. Journal of Elementology, 24(2), 711-726.

Sutherland, M. A., Bryer, P. J., Davis, B. L., Smith, J. F., McGlone, J. J. (2012). The combined effects of transport and food and water deprivation on the physiology of breeding age gilts. Livestock Science, 144, 124–131.

Torres, T. R., Do Ludke, M., Ludke, J. V., Dos Santos, M. J. B., Lima, M. R., & Dos Santos, P. A. (2013). Performance of broilers fed during 21 days on mash or pellet diets containing whole or ground pearl millet grain. Brazilian Journal of Poultry Science, 15(4), 371-378.

Upadhaya, S.D., Yun, H.M. and Kim, I.H., 2016. Influence of low or high density corn and soybean meal-based diets and protease supplementation on growth performance, apparent digestibility, blood characteristics and noxious gas emission of fattening pigs. Animal Feed Science and Technology, 216, pp.281-287.

Xie, C., Zhang, S., Zhang, G., Zhang, F., Chu, L. and Qiao, S., (2013). Estimation of the optimal ratio of standardized ileal digestible threonine to lysine for fattening barrows fed low crude protein diets. Asian-Australasian Journal of Animal Sciences, 26(8), 1172–1180.

Yin, Y. L., Gurung, N. K., Jeaurond, E. A., Sharpe, P. H., & de Lange, C. F. M. (2002). Digestible energy and amino acid contents in Canadian varieties of sorghum, pearl millet, high-oil corn, high-oil–high-protein corn and regular corn samples for growing pigs. Canadian Journal of Animal Science, 82(3), 385-391.

Zielonka, L., Przewoski, W., Gajecka, M., Jakimiuk, E., & Gajecki, M. (2010). The effect of exogenous proteases on selected hematological and serum biochemical parameters in boars. Polish Journal of Veterinary Sciences, 13(1), 151-156.