

# Effect of Dietary Extruded Linseed and Walnut Meal Mixture (8:1) on Performance and Plasma Protein Profile in Weaned Piglets

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## Abstract

The effects of dietary extruded linseed (ELS):walnut meal (WM) mixture (8:1) on performance and plasma protein profile in weaned piglets was evaluated for 21 d. Topigs piglets (n=40; BW=8.02±0.82 kg), age 30±3 days, were allotted into 2 groups and fed 2 diets: control [C, based on corn-triticale-soybean meal (SBM)] and experimental (ELS:WM, where the ELS:WM mixture (8:1) partially replace SBM). Blood samples were collected at 7d and 21d after weaning. The plasma protein profile (total protein, total bilirubin, albumin, creatinine, uric acid, urea nitrogen-BUN) were determined by a chemistry analyser. Dietary ELS:WM mixture improve the BW (P=0.047) and ADG (P=0.036) of piglets at 21d after weaning vs C, whereas ADFI and F:G ratio were increase (P>0.05). The plasma protein profile of piglets fed dietary mixture was not affected at 7d and 21d after weaning, except plasma BUN concentration that was decrease (P=0.027) at 21d after weaning. Lower BUN concentration indicated higher availability of dietary nitrogen reflected in a higher deposition of protein. We concluded that dietary ELS:WM mixture improve piglets performance and positively affect plasma protein profile, especially BUN in weaning period.

**Keywords:** extruded linseed:walnut meal, performance, plasma parameters, weaned piglets

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## INTRODUCTION

In modern pig production, weaning is still associated with nutritional, environmental and immune challenges that may lead to considerable economic losses to pork producers (Li *et al.*, 2014; Weng, 2017). Generally, weaning period is characterized by decreased feed intake, altered gut integrity and increased concentrations of inflammatory markers in blood (Le Dividich and Sève, 2000; Pié *et al.*, 2004; Montagne *et al.*, 2007).

The effects of dietary protein sources on growth and health of piglets have been studied extensively (Pluske *et al.*, 2002; Kim *et al.*, 2006; Wang *et al.*, 2007; Hermes, 2011; Habeanu *et al.*, 2015), but there is still a lack of information on the

effect of dietary proteaginous sources (e.g. linseed, flaxseed, walnut by-products) or their mixture, rich sources of essential polyunsaturated fatty acids (PUFAs), which could help piglets to adapt to the rapidly changed diet during weaning (Rodriguez-Leyva *et al.*, 2010; Li *et al.*, 2014). Due to the rich content in alpha-linolenic acid (ALA) and lignans which have antimicrobial properties linseed is unique as a vegetable source (Kiarie *et al.*, 2014). Walnut meal could be a valuable alternative to soybean meal, besides having a high protein level, is also a rich source of bioactive compounds (ALA, antioxidants, dietary fiber) with potential benefit on piglets health status. A mixture between linseed and walnut meal (8:1) could be a good opportunity

**Table 1.** Ingredient and nutrient composition of piglets diets

Ingredients, %	Control C	Experimental ELS:WM
Corn	44.11	40.93
Triticale	25.00	25.00
Extruded linseed:walnut meal (8:1)	-	9.00
Soybean meal	18.00	13.00
Corn gluten	3.00	3.00
Milk replacer	5.00	5.00
Vegetable oil	1.50	0.50
DL-Methionine	0.04	0.11
L-Lysine	0.34	0.52
Calcium carbonate	1.70	1.72
Monocalcium phosphate	0.10	0.01
Phytase	0.01	0.01
Salt	0.10	0.10
Choline premix	0.10	0.10
Vitamin-mineral premix <sup>1</sup>	1.00	1.00
Analysed composition, %		
Dry matter	89.52	89.19
Crude protein	18.75	18.33
Lysine	1.20	1.20
Methionine+Cystine	0.72	0.72
Calcium	0.90	0.90
Phosphorus	0.78	0.78
Crude fiber	4.75	4.73
Crude fat	3.69	4.30
Metabolisable energy ME (kcal/kg) <sup>2</sup>	3260.7	3256.9

Note: <sup>1</sup>Vitamin mineral premix added at 1% to the diet contained (per kg feed): 10000 IU vitamin A; 2000 IU vitamin D3; 30 IU vitamin E; 3 mg vitamin K3; 2 mg vitamin B1; 6 mg vitamin B2; 20 mg vitamin B3; 13.5 mg vitamin B5; 3 mg vitamin B6; 0.06 mg vitamin B7; 0.8 mg vitamin B9; 0.05 mg vitamin B12; 10 mg vitamin C; 30 mg of Mn; 110 mg of Fe; 25 mg Cu; 100 mg Zn; 0.38 mg I; 0.36 mg Se; 0.3 mg Co; 60 mg antioxidant. <sup>2</sup>ME calculated based of feed ingredients composition using regression equations (NRC, 1998).

to improve the energy, amino acids and the fatty acid content of the compound feed.

The complementary effect of dietary proteaginous mixture on plasma protein profile in piglets is still little known, therefore this study was conducted to evaluate the effects of dietary extruded linseed: walnut meal mixture (8:1) on growth performance and plasma protein profile in weaned piglets for 21 d.

## MATERIAL AND METHODS

The animals were treated in accordance with EU Directive 2010/63/EU (OJEU, 2010) and all experimental procedure was approved by the

Animal Care Committee of the National Research-Development Institute for Animal Biology and Nutrition (Balotesti, Romania).

*Animals and experimental design.* Forty weaned piglets Topigs [♀ Large White x Hybride (Large White x Pietrain) × ♂ Talent, mainly Duroc], average body weight (BW) 8.02±0.82 kg, age 30±3 days, were randomly divided into 2 groups with 2 replicate each. Piglets were fed with 2 diets: control [C, based on corn-triticale-soybean meal (SBM)] and experimental (ELS:WM, where the mixture of extruded linseed:walnut meal (8:1) replaced 27.8% of SBM) for 21d. The diets (Tab. 1) were isocaloric and isoenergetic, formulated to meet

**Table 2.** Chemical composition of feed ingredient and dietary mixture used in piglets diets

Item, %	Extruded linseed	Walnut meal	Dietary mixture ELS:WM (8:1)
Dry matter	91.09	90.91	91.00
Crude protein	18.26	36.53	27.40
Crude fat	21.18	7.42	14.30
Crude fiber	17.04	14.07	15.56
ME, kcal/kg <sup>1</sup>	3970	3174	3572

Note: <sup>1</sup>ME calculated based of feed ingredients/dietary mixture composition using regression equations (NRC, 1998).

**Table 3.** Growth performance of piglets fed dietary ELS:WM mixture (8:1)

Item	Control diet	Experimental diet	SEM	<i>P</i> -value
BW at weaning, kg	7.93	8.07	0.14	0.662
BW at 21d after weaning, kg	12.21 <sup>b</sup>	13.53 <sup>a</sup>	0.03	0.047
ADG, g	0.214 <sup>b</sup>	0.235 <sup>a</sup>	0.005	0.036
ADFI, g	0.370	0.420	0.08	0.130
F:G ratio, g feed:g gain	1.73	1.79	0.07	0.128

Note: BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; SEM, standard error of the mean; Different letters between column denote significant differences (Tukey test,  $p < 0.05$ ).

similar content of essential aminoacids (lysine and methionine), calcium and phosphorus, in accordance with nutrient requirements of hybrid (Topigs, 2012). The basal chemical composition of extruded linseed, walnut meal and the dietary mixture used in this study are presented in Tab. 2. Feed and water were given *ad libitum* to piglets for all experimental period.

The growth performance parameters were evaluated: body weight (BW) at weaning and at 21d after weaning, feed intake (FI), from which average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (F:G ratio) were calculated.

**Chemical and biochemical analyses.** The chemical composition of feed ingredients and compound feeds samples were determined using standardized methods (OJEU, 2009).

Blood samples were collected from 8 piglets per group (4 male and 4 female) at 7d and 21d after weaning by jugular venipuncture in heparinized vacutainer tubes (6 mL). After blood samples were centrifuged (3000 rpm for 15 min.) the plasma protein profile (total protein, T-Pro; total

bilirubin, T-Bil; albumin, Alb; creatinine, Cre; uric acid, UA; urea nitrogen, BUN) were determined by a chemistry analyser (Spotchem EZ SP-4430, Arkray, Japan) using commercial kits.

**Statistical analysis.** The data obtained were analysed using the GLM procedure (SPSS, 2011). One-way analysis of variance (ANOVA) with the Tukey's comparison test was used to evaluate statistical significance of differences between dietary treatments. The results are expressed as means and standard error of the mean (SEM). Differences were considered significant at  $P \leq 0.05$ . Replicate was considered as experimental unit in case of growth performance and each individual for plasma parameters determinations.

## RESULTS AND DISCUSSION

**Growth performance.** Table 3 presents the average body weight, average daily gain, average daily feed intake and feed conversion ratio of piglets fed dietary extruded linseed:walnut meal mixture (8:1). Our results shown that piglets fed dietary mixture had higher BW (+10.81%;  $P=0.047$ ) and ADG (+9.81%;  $P=0.036$ ) at 21d after

**Table 4.** Plasma protein profile at 7d and 21d after weaning of piglets fed dietary ELS:WM mixture (8:1)

Parameters	References value*	Day	Control diet	Experimental diet	SEM	P-value
		7	21	7		
T-Pro, g/dl	5.8-8.3	7	4.98	4.75	0.09	0.269
		21	5.4	5.18	0.11	0.379
Alb, g/dl	2.3-4.0	7	2.75	2.76	0.06	0.927
		21	3.13	3.03	0.08	0.595
T-Bil, mg/dl	0-0.5	7	0.25	0.30	0.02	0.275
		21	0.25	0.29	0.02	0.348
Cre, mg/dl	0.8-2.3	7	1.40	1.30	0.04	0.188
		21	1.32	1.20	0.03	0.418
UA, mg/dl		7	0.68	0.65	0.01	0.454
		21	0.63	0.62	0.02	1.00
BUN, mg/dl	8.2-25	7	14.75	13.44	0.70	0.129
		21	13.98 <sup>a</sup>	11.89 <sup>b</sup>	0.60	0.027

Note: \*Merck Veterinary Manual (2010). Tenth Edition. Merck &Co. Inc., USA; T-Pro, total protein; Alb, albumin; T-Bil, total bilirubin; Cre, creatinine; UA, uric acid; BUN, urea nitrogen; SEM, standard error of the mean. Different letters between column denote significant differences (Tukey test,  $p < 0.05$ ).

weaning compared with C, whereas ADFI and F:G ratio were insignificantly increase ( $P > 0.05$ ). The improvement in growth performance of piglets could be attributed to the anti-inflammatory properties of n-3 PUFA and bioactive compounds with benefits on health status contained by dietary extruded linseed:walnut meal mixture and confirmed that dietary mixture represent a viable alternative that could partially replace the soybean meal in piglet diets.

The effects of feeding linseed was extensively studied on grower-finisher pigs and had been stated that linseed is an effective feed for increasing the n-3 PUFA content of pig meat and improve the n-6:n-3 PUFA ratio (Rentfrow *et al.*, 2003). Previous findings (Matthews *et al.*, 2000; Riley *et al.*, 2000; Kouba *et al.*, 2003; Corino *et al.*, 2008; Guillevic *et al.*, 2009; Bečková and Václavková, 2010; Nurnberg *et al.*, 2011) reported that inclusion of linseed in pig diets may improve the nutritional value of pork meat without deleteriously affecting organoleptic characteristics, oxidation or colour stability and enhance the levels of n-3 fatty acids, which have a potentially positive health effect in humans.

However, from our knowledge little scientific literature are available about the effect of feeding linseed or dietary extruded linseed mixture on performance of piglets for comparison. Quiniou *et*

*al.* (2010) evaluated the effect of extruded linseed incorporation in diets of sows' (3.5%) and/or pigs' (2%) on both performance of sows and their progeny. The authors stated that the addition of extruded linseed significantly faster the farrowing progress, the survival rate of piglets weighing between 1.0 and 1.4 kg at birth was significantly improved and pig's performance (daily feed intake, feed conversion ratio, or carcass fatness) from 24 to 110 kg BW were significantly influenced by the treatment. Quiniou *et al.* (2010) observed during the growing phase a significant higher ADG in pigs fed extruded linseed and suggested that more investigations would be required in order to determine whether this improved growth rate would result from a better adaptation capacity to new breeding conditions induced by the anti-inflammatory properties of n-3 PUFA stored in pigs during gestation and lactation, or not.

Recently, Habeau *et al.* (2017) reported that dietary peas:linseed mixture (3:1) did not significantly affect the growth performance, lipid and protein biochemical profile, and suggested that the combination of these valuable vegetable sources give the opportunity to optimize diet for weaning piglets.

*Biochemical parameters.* The effects of dietary mixture of extruded linseed:walnut meal

on plasma protein profile of piglets at 7 and 21d after weaning are given in Tab. 4. The growth performance of piglets are affected by the metabolic efficiency reflected by level of plasma parameters value that are considered important markers for health status (Habeanu *et al.*, 2011; Yeom *et al.*, 2012).

Our results shown that at 7d after weaning plasma protein profile of piglets fed dietary mixture was not affected, the concentrations of T-Pro, Alb, T-Bil, Cre, UA and BUN were similar between treatments ( $P>0.05$ ) and range in normal limits (Merck Veterinary Manual, 2010).

There were no difference in plasma protein profile at 21d after weaning, except the plasma urea nitrogen (BUN) concentration that significantly decreased with 14.95% ( $P=0.027$ ) as result of feeding dietary mixture.

Plasma urea is considered an important marker of protein utilization efficiency (Heo *et al.*, 2009; Kim *et al.*, 2012). Lower concentration of BUN indicated higher availability of dietary nitrogen (Figueroa *et al.*, 2002; Shen *et al.*, 2012) reflected in a higher deposition of protein, which may explain the better performance response of piglets fed dietary mixture.

It is widely known that BUN and creatinine (a non-protein nitrogenous compound produced by the breakdown of creatine in muscle), are indicators of renal function, but creatinine (Cre) it is less influenced by other factors such as diet and hydration (Washington and Van Hoosier, 2012).

The concentrations of BUN and Cre obtained in our study were between normal values: 8.2-25 mg/dl for BUN and 0.8-2.3 mg/dl for Cre (Merck Veterinary Manual, 2010). According to Washington and Van Hoosier, (2012) a normal BUN:Cre ratio is 10–20 mg/dl to 1 mg/dl, and an increased or decreased BUN:Cre ratio indicates renal disorder. Our results shown that the dietary treatments did not influence the BUN:Cre ratio at 7 and 21d after weaning, the values obtained were in normal limits ( $>10$  mg/dl). Previously, Habeanu *et al.*, (2017) noted that fed piglets with a peas: linseed (3:1) mixture had no significant effect on plasma protein profile and BUN:Cre ratio at 21d after weaning.

## CONCLUSION

Our results suggest that dietary extruded linseed: walnut meal mixture (8:1) improve growth

performance and has a positive effect on biochemical parameters, especially on urea nitrogen of piglets in weaning period and could represent a good alternative that partially replace the soybean meal in piglets.

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