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N-3 PUFA-ENRICHED HEMP SEED DIET MODIFIES BENEFICIALLY SOW MILK COMPOSITION AND PIGLETS' PERFORMANCES

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Abstract

An experiment was conducted to evaluate the dietary hemp seed (HS) influence on sow milk composition and piglets' performances. Ten sows were assigned randomly, to a control group (CL) with classical diets, and an experimental group (HSL) fed with 5% HS. Their litter (N=96) were divided in two groups: HH/CH with 1.5% HS and CC/HC without HS. The HS antioxidant activity was determined by DPPH method, phenolic compound by Folin–Ciocalteu, cannabinoid by colorimetry. Milk and colostrum samples from sow were collected manually. Gas chromatography was used to determine milk fatty acids (FA) composition. Colostrum and sow milk content of protein was determined by biuret reaction and lactose concentration was determined using phosphomolybdenic reagent. The lipid specimen was heated with concentrated sulfuric acid. Then vanillin and phosphoric acid are added to yield a pink coloured product which has been measured at 530 nm and quantified using a triolein standard. Milk yield (MY) was calculated. Average daily gain (ADG) of piglets at 0 to 7 d (days) was higher (P= 0.001) for HC/HH group and tended to be higher for HC/ HH group at 0-21 d. Milk yield calculated at peak lactation was > 2.1-fold in HSL group than in CL group. The diets tend to influence milk fat content, whereas the day had a significantly higher influence for protein and lactose. The n-3-rich diet favours milk deposition of α-linolenic FA (ALA), linoleic FA (LA) and total n-3 polyunsaturated FA (PUFA). This led to a significantly lower n-6:n-3 ratio in milk of HSL group. Docosahexaenoic FA (DHA) increased 1.87 and 2.33-fold at 0-7 d and 0-21 d in HSL group. We can conclude that HS altered beneficially milk FA associated with litter performance. Significantly positively correlated with MY, milk constituents declined in time.

Key words: *hemp seeds, milk composition, milk yield, sows, piglets performances*

INTRODUCTION

The industrial hemp (*Cannabis sativa)* is an ideal source of n-3 and n-6 FA, whose ratio is ideal for health. Although this ingredient has been used for many years, the studies on its physiological effects are limited [8]. The researches show that n-6:n-3 ratios below 2:1 are beneficial to human health [26] [16]. The dietary ingredients rich in essential fatty acids (EFA), are known to improve the survival rate of the new-born piglets and the sow milk production [3]. The milk, as main supplier of nutrients for the piglets in their early days of life [22], plays an essential role in the rate of survival and in the development of the piglets [11]. Fat, lactose, protein and minerals are the major components of the milk. Furthermore, α-linolenic FA (ALA) and linoleic FA (LA) are critical to the formation of tissues and to the regulation of the immune functions [19] [18]. Even though the current scientific information is rather advanced regarding the beneficial effects of the EFA, many gaps still exist in the knowledge about the effect of less and/or unknown dietary resources on the relation between the various production parameters in sows and piglets. This research paper addresses the hypothesis that the dietary hemp seed (HS) could modulate fatty acids (FA) of sow milk composition and the associated performances. The aim of this study was to evaluate sows milk content of protein, lactose, fat and the FA. The protein, fat and lactose value were compared with the theoretical calculated values of these parameters using the experimental evidences from our study and the equations from the literature [12] [30].

MATERIALS AND METHODS

Animal care

The research protocol has been approved by the Ethics Committee within INCDBNA Balotesti. It was developed according to Law 43/2014, Romania, regarding the protection of animals used for scientific purposes. The experiment was carried out in 2016-2017, to experimental station of INCDBNA Balotesti.

Animal and Diet

The experiment was conducted with 96 suckling piglets from 10 multiparous sows Topigs [♀ Large White × Hybrid (Large White × Pietrain) × ♂ Talent, mainly Duroc]. A total of 123 piglets were born, of which 11 stillbirths; another 16 piglets died throughout the experimental period. The lactating sows were assigned randomly for 21 d (days) to two groups: control (CL), which received the classical diets, and experimental (HSL for lactating sows), treated with 5% HS *Jubileu* variety (Table 1).

Table 1. Composition of the experimental diets for pregnant, lactation sow and nursery piglets (as- fed basis)

Items, g kg^{-1}	**Lactating sows**		**Piglets**	
			CL sow	HSL sows
	CL	HSL	CC/HC	HC/HH
Corn	562.5	553.7	648.0	641.2
Rice flour	100.0	100.0	-	-
Jubileu hemp seeds	-	50.0	-	15.0
Soybean meal	180.0	150.0	220.0	215.0
Sunflower meal	100.0	100.0	-	-
Corn gluten	-	-	30	30
Milk replacer	-	-	50	50
Onix oil	16.0	5.0	8.0	5.0
DL-methionine	-	-	0.9	0.9
L-lysine	0.4	0.9	3.1	3.1
Calcium carbonate	19.5	19.4	14.6	14.9
Monocalcium phosphate	5.6	5.0	13.4	12.9
Salt	4.0	4.0	1.0	1.0
Choline premix	2.0	2.0	1.0	1.0
Vitamin-mineral premix $^{(P1+2)}$ ‡	-	-	10.0	10.0
Vitamin-mineral premix $^{(P5+6)}$ ‡	10.0	10.0	-	-
Analysed composition (g kg^{-1})				
Dry matter	895.4	893.9	887.9	890.4
ME (MJ /kg) †	12.76	12.75	13.73	13.74
Crude protein	169.8	170.2	195.9	197.9
Lysine†	8.7	8.7	12.0	12.0
Met + Cys†	6.3	6.3	7.2	7.2
Calcium	9.5	9.6	9.7	9.5
Phosphorus	6.0	6.1	6.5	6.5
Cellulose	63.1	76.9	40.5	43.6
Fat	43.1	46.5	27.5	28.2

† ME and amino acid contents were calculated based on feed composition. Gestation diets were provided in one meal / day; lactation diets were provided in two meals /day.

‡Vitamin mineral premix added at 1% to the diet contained (/kg feed): P1+2: 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. Vitamin mineral premix P5+6: 9000 IU vitamin A; 1500 IU vitamin D3; 50 IU vitamin E; 2 mg vitamin K3; 1.5 mg vitamin B1; 5.2 mg vitamin B2; 15 mg vitamin B3; 8.1 mg vitamin B5; 2 mg vitamin B6; 0.10 mg vitamin B7; 0.5 mg vitamin B9; 0.03 mg vitamin B12; 39 mg of Mn; 100 mg of Fe; 15 mg Cu; 100 mg Zn; 0.3 mg I; 0.22 mg Se; 0.25 mg Co; 60 mg antioxidant

Source: compound feed formula calculated, Mihaela Habeanu, INCDBNA Balotesti

The piglets from the two groups of sows were assigned randomly to two groups, so that every sow group had two litter groups (with and without HS dietary addition, Fig 1).

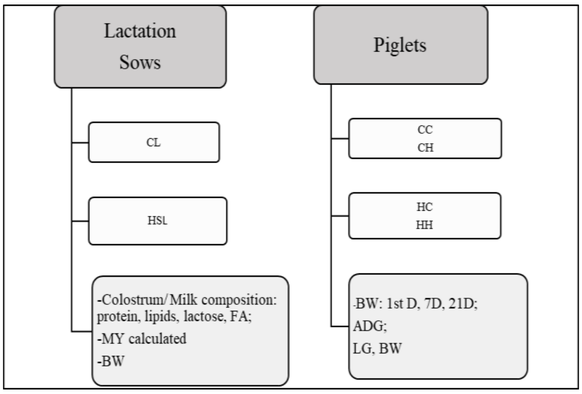

Fig. 1. Experimental design
Source: original, Habeanu protocol

The piglets were kept within the same litter after farrowing (AF). The HS was ground and screened through 8 mm mash sieves and analysed chemically. Ten day AF piglets started to receive pelleted feed *ad libitum.*

Assay Procedures and Analyses

In the first day AF (within the interval of ~ 12h), at 7 d and 21 d AF, the sows milk samples were collected. At 114 d of pregnancy, the sows received intramuscularly 1 mL D-cloprostenol (75μg active substance /mL), so as to farrow as a group. The sows had free access to the pelleted diets, and the leftovers were recorded on a daily basis. Piglets were weighed at 1st, 7 d and 21 d AF.

Standardized methods, as per Commission Regulation (EC) no. 152 (2009), were used to determine the gross chemical composition. Crude protein was determined by the Kjeldal method, on the basis of the nitrogen content. The crude fat was determined by continuous extraction in organic solvents followed by fat measurement with Soxhlet, after solvent removal. The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method. The metabolisable energy (ME) was calculated using the regression equations developed by the „Oskar Kellner" Institute of Animal Nutrition:

$$ME = 5.01 \times DP + 8.93\ EE + 3.44\ CF + 4.08\ DNFE.$$

The antioxidant activity of the HS was determined by the DPPH method according to Arnous et al. [2] with slight modifications. The polyphenols from HS were extracted in acetone 80%, ethanol and boiled water (1:7 w/v sample: solvent ratio) for 24 hours at 37^{o}C under continuous shaking according to the previous study [4]. The total phenolic compound of the extract was determined by the Folin–Ciocalteu method, adapted to a microscale, after method described by Arnous et al. [2]. The results were expressed as gallic acid equivalents (GAE)/L. The cannabinoid content was determined by the colorimetric method, which consists of a colour scale, with grades from 1 to 10, corresponding to 0 – 1.0% THC content.

Milk and colostrum samples from sow were collected manually, according to the method described by Noblet and Etienne [21]. The samples were stored at -80^{o}C until assayed, to inactivate different constitutive enzymes and avoid biochemical markers alteration or hydrolysis. On 7 and 21 d of lactation, piglets were separated for two hours from the sow, after the morning suckling, and milk samples were collected. After about 70 min. the sows were injected with 10 IU oxytocin (Veyx-Pharma, Germany) in the auricular vein, after which they were milked by hand. Milk was collected from all functional mammary glands.

The milk composition (protein, fat and lactose)

The lipid concentration was determined using the method described previously by Knight et al. [17], Al-Mashhedy et al. [1]. In this procedure, the lipid specimen was heated with concentrated sulfuric acid. Then vanillin and phosphoric acid are added to yield a pink coloured product which has been measured at 530 nm and quantified using a triolein standard. Colostrum and sow milk content of protein was determined by biuret reaction. Prior to the protein determination, the colostrum and milk was defatted by centrifuging samples upside down in a tightly sealed polypropylene tube at 3000 g and at 4°C for 15 minutes. Biuret reagent containing 18 mmol disodium ethylenediaminetetraacetate, 15 mmol Cu^{2+}, and 1 mol of NaOH per litre was prepared as described by Chromy and Ficher [5] and Chromy et al. [6]. A sample of 50 μL defatted milk was mixed with 1,000 μL of the biuret reagent at room temperature and the absorbance was read at 550 nm after 30 minutes taking into account the interference due by the abundant presence of lactose. Further, milk and colostrum lactose concentration was determined using phosphomolybdenic reagent essentially described by Hindin [15] using lactose 1% as standard.

The fatty acids composition of colostrum, milk, HS and diets were determined by gas chromatography using a Perkin Elmer-Clarus 500 gas chromatograph (Massachusetts, United States), fitted with Flame Ionization Detector (FID) and capillary separation column with high polar stationary phase Agilent J&WGC Columns, (United States), DB-23 dimensions 60m × 0.250 mm × 0.25 μm. The FA were identified by comparison with blank chromatograms and were subsequently determined quantitatively as percent of total FAME. SUPELCO 37 component FAME Mix was used; 10 mg/ml as standard solution of methylated FAs and also Soybean Oil and Sunflower Oil; SUPELCO, as reference material was used. We used hydrogen as carrier gas and the air oxygen as burning gas, method described by Hăbeanu et al. [13].

Statistical analyses and calculation

All experimental results obtained were submitted to variance analysis using by SPSS - general linear model (Statistics version 20, 2011). The response to the dietary treatment was variable dependent, and the diet and/or sampling day were fixed factors. The results were expressed as mean value and standard error of the mean (SEM). Pearson's correlation (r) was used to evaluate the linear dependence between different parameters. We used the Equation (Eq.) described by Hansen et al., [12], Wood [30] to calculate milk yield (MY):

MY(t) = a x tb x exp (c x t)

where:

MY(t) = milk yield , t = time AF;

a, b, c, = coefficients described by Woods, [30].

RESULTS AND DISCUSSIONS

Results

Hempseed and diet characteristics

Characterized by higher protein quality, free of trypsin inhibitors and highly digestible compared to other vegetal sources [14] hemp is a plant with valuable potential for livestock. The proximate analysis of HS *Jubileu* used in our study was*:* 89.67% dry matter: 21.26% protein, 27.70% fat; 28.82% cellulose. HS had the THC content of 0.0139%, lower than that noticed by EFSA Regulation [27]. The total polyphenols content was 10.57 ± 0.12g GAE/ 1kg seed (or 2.93 mg GAE / g oil) compared to 0.44 mg GAE/g oil noticed by Yu et al. [29] in the cold-pressed HS oil extract. The antioxidant activity expressed as antiradical activity was 83.58 ± 2.36 mM TE (trolox equivalents)/g seeds.

The FA composition of the HS, reveals the predominance of PUFA (72.58%) and n-6:n-3 PUFA ratio of 3.19. ALA, as predominant n-3 FA, had a concentration of 17.06%, LA belonging to n-6 family, had a concentration of 53.79%, the oleic FA as main monounsaturated FA (MUFA) had a level of 14.46%, and the main saturated (SAT) FA, palmitic FA was 7.18%. The favourable FA composition of the HS reflected in the diet (Table 2).

The 5% dietary HS given to HSL lactating sows increased 1.61 times ALA concentration in the diet, 1.01 times LA concentration, while reducing 1.52 times n-6:n-3 ratio compared to CL group. In piglets, 1.5% dietary HS given to groups CH/HH, increased 1.40 times ALA concentration, while decreasing 1.52 times n-6:n-3 ratio compared to CC/HC feed group.

The sows had an average body weight 213 Kg ± 38 AF. The average compound feeds intake during lactation was closed between groups: CL sows had an average daily feed intake (ADFI) of 5.16 Kg, while the sows with HS in the compound feed consumed 5.57 Kg/day. The daily intake of protein and fat was calculated from the daily feed intake and from the protein and fat feed level.

Table 2. Centesimal FA composition of diets

Fatty acids†, % of total FAME	Sow		Piglets	
	CL	HSL	CC/HC	CH/ HH
C14:0	0.24	0.21	0.54	0.51
C16:0	15.02	14.09	12.99	12.81
C16:1	0.21	0.23	0.11	0.22
C18:0	2.43	2.41	2.93	2.82
C18:1n-9	31.99	29.41	26.86	25.52
C18:2n-6 (LA)	44.96	45.53	51.63	50.76
C18:3n-3 (ALA)	3.8	6.12	3.83	5.37
C18:4n-3	0.49	0.54	-	0.35
C20:0	-	0.71	-	0.44
C20:2n-6	0.51	0.42	0.23	0.24
C20:3n-6	0.08	0.05	-	-
C20:3n-3	0.06	-	-	-
C20:4n-6	0.22	0.04	-	-
Σ PUFA	50.12	52.7	55.68	56.73
Σ n-6	45.77	46.04	51.86	51.01
Σ n-3	4.35	6.66	3.83	5.73
n-6:n:3	10.52	6.91	13.55	8.9
Σ MUFA	32.2	29.64	26.97	25.71
Σ SAT	17.69	17.57	17.35	17.53

†ALA –α-linolenic FA; LA – Linoleic FA; PUFA – polyunsaturated FA; MUFA – monounsaturated FA.
Source: analyses made in the Chemistry Lab, INCDBNA

Throughout the lactation period, the sows had the following daily intakes of protein and fat: group CL consumed 876 g protein and 222 g fat, while the group treated with HS consumed 948 g protein and 259 g fat.

Table 3 shows litter performance. The average LS was 10 in HSL sows group, and 9.25 in CL sows group. The average initial BW of the piglets was similar among the groups, but after 7 d a significant increase was noticed in group from HS sows compared to piglets from C sows (>1.18 times). After 21 d we noticed a tendency the treatment had significantly effect. Piglets ADG 7 d AF was greater in HC/HH group (1.17-fold higher than in the sows group with no HS treatment, P = 0.001), whereas to 21 d tended to be greater ($P < 0.10$) for HC/ HH group. Litter weight did not differ significantly between groups (1.18 higher in HC/HH group than in CC/CH group); however, the day's effect was very significant irrespective of the treatment.

Calculated MY and lactation curve

MY was calculated according to Eq. described by Hansen et al. [12] for 7 d up to 21 d (Table 4). MY was 1.13-fold higher in HSL group compared to CL group. In our study, the MY values were similar to those reported by Hansen et al. [12] in case of HS dietary addition, while in the group fed classically they were 15.6% lower than the data reported by Hansen et al. [12]. While the MY minimum value was similar between the two groups, the MY maximum value was higher in the group with dietary HS addition. The mean MY at peak lactation was 8.58 Kg (<7% than value of Hansen et al. [12] 11% than value reported by Daza & Centeno [7].

Table 3. Body weight and average daily gain of the nursery piglets

Items†	CL		*Mean*	HSL		*Mean*	*SEM*	*P- value*
	CC	CH		HC	HH			
BW 1th AF, Kg	1.36	1.68	*1.52*	1.61	1.45	*1.53*	*0.05*	*T*
BW 7 d, Kg	2.19	2.58	*2.39*	2.95	2.67	*2.81*	*0.07*	**
BW 21 d, Kg	4.73	5.38	*5.08*	5.53	5.45	*5.49*	*0.11*	*T*
ADG 7 d, Kg	0.119	0.129	*0.124*	0.188	0.175	*0.181*	*0.006*	**
ADG 21 d, Kg	0.161	0.177	*0.169*	0.187	0.191	*0.189*	*0.004*	*T*
Total born			*11.75*			*12.0*		
LS§	8.5	10	*9.25*	10	10	*10.0*		
LG (diet effect)			*1.46*			*1.90*	*0.10*	*NS*
LW, 7 d			*22.18*			*27.98*	*3.06*	*NS*
LW, 21 d			*47.06*			*54.92*	*4.75*	*NS*

† Diet of piglets were classical (CC and HC - piglets from sows fed with and without HS) and 1.5% HS supplementation (CH and HH – piglets from sows fed with and without HS dietary addition) ; ADG average daily gain; litter weight LW; litter gain LG; litter size LS.
**Highly significant effect; NS not significant; T = trend that the treatment has effect;
Source: Results of *in vivo* biological test, Habeanu

The results were used to plot the sow lactation curve (Figure 2). In our study, MY increased up to 19-20 d, when the peak of lactation occurred, then declined.

Milk composition

The composition of colostrum, transition milk and mature milk are different. The comparison between values calculated and determined through analyses are shown in Table 5. While protein values analysed are higher than the calculated ones, whatever the treatment or day, both for fat and lactose the evaluated values are lower. The treatment tend to influence milk fat content (P = 0.06), whereas the day had a significantly higher influence for protein and lactose milk content. By dietary addition of the HS the recorded values were higher than those of CL group.

Table 4. Milk yield calculated by theoretical Eq. The effect of the dietary treatment and day, %

Group†	**MY**	**MY at peak lactation (Kg)**	**MY at 7 d**	**MY at 21 d**
CL	7.35	7.98	7.04	7.95
HSL	8.31	9.17	7.82	9.09

† Diets of sow were classical (CL) and 5% HS supplementation (HCL).
Source: date calculated using regression Eq, Habeanu & Surdu

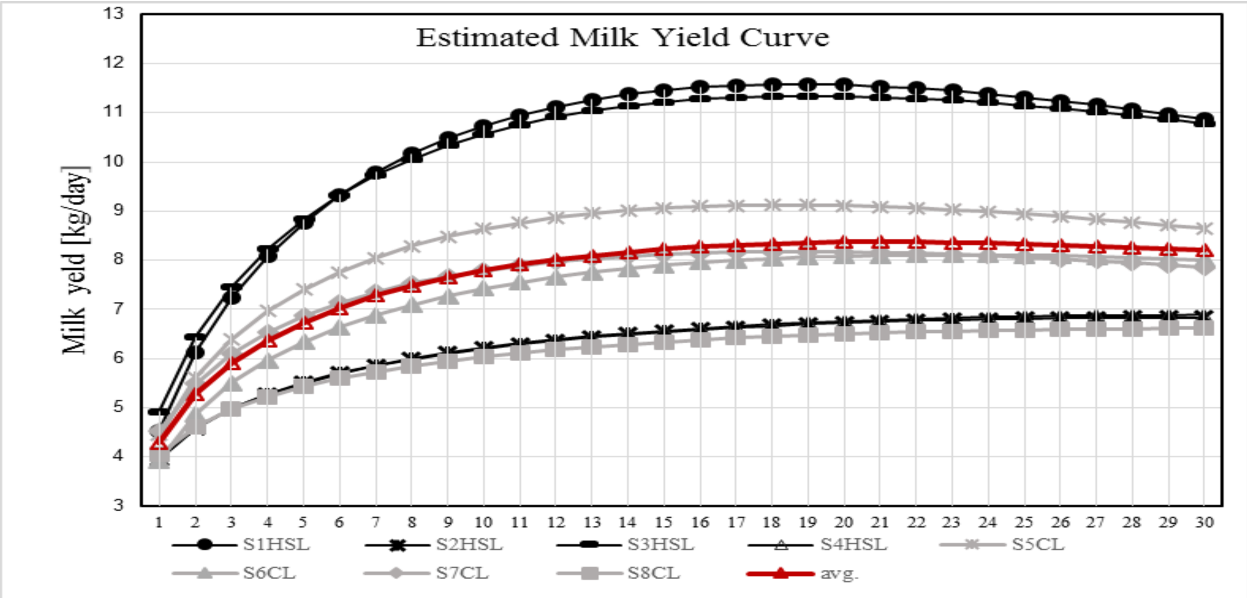

Fig. 2. Estimation milk yield curve.
Lactation curve estimated for a period of 30 days, using the Eq. of Hansen *et al*., (2012). Each line represents a sow. Milk yield reach the peak between 19-20 d.
†HSL – lactation hemp seed group; CL – lactation control group.
Source: original, MY curve calculated, Habeanu & Surdu

Table 6 shows the centesimal sow milk and colostrum FA composition. The dietary addition of HS in lactating sows diets improved milk EFA composition. In our study, the dietary EFA composition differed according to the source of dietary lipids, which was reflected in milk composition. Thus, significant differences were noticed for ALA and LA as predominant EFA. While LA content slightly increased following the HS treatment, both at 7 d and at 21 d, these n-6 FA decreased slightly compared to CL group. On the other hand, ALA increased highly significantly both in colostrum and milk, following the HS treatment (1.29-fold in the colostrum, and 1.58-fold in the milk at 7 d and 1.47-fold at 21 d).

The higher ALA concentration, like in the diet, produced a significantly higher level of n-3 FA and, implicitly, a lower n-6:n-3, at 21 d, to the same proportion as in the diet. One must also notice the much higher level of milk docosahexaenoic (DHA) in HSL group (1.87-fold at 7 d and 2.33-fold at 21 d), the differences were not, however, significantly. In line with the hypothesis that ALA is ingested by the piglets via milk [25] another long-chain FA, EPA was noticed in low proportions in the colostrum and in the milk collected at 7 d, only from group HSL which supports the conclusion of Sampels et al. [25] that eicosapentaenoic (EPA) too is transferred via milk, and probably synthesized from ALA.

Table 5. Comparative milk content as effect of the dietary treatment and day, %

Items	CL		HSL		*P value*	
	Mean value				Diet	Day
	Calculated †	Evaluated	Calculated†	Evaluated		
Protein					0.94	<0.0001
12h AF		9.06		8.99		
7D	5.42	5.73	5.42	6.06		
21D	5.0	5.36	5.0	5.48		
Fat					0.06[T]	0.68
12h AF		6.01		6.94		
7D7	7.71	6.03	7.71	6.43		
21D	6.8	5.9	6.8	6.26		
Lactose					0.97	<0.0001
12h AF		2.74		2.72		
7D7	5.47	4.75	5.47	4.70		
21D	5.33	4.52	5.33	4.35		

†Milk content of protein, lipids and lactose were calculated using Hansen *et al.*, (2012) equation

Source: analyses made at University of Bucharest, Panait, Stoian, Gheorghe, Lefter; Estimated values, Habeanu

Table 6. Fatty acids composition of the colostrum/milk (1st AF, 7 d, 21 d) due to the dietary *Jubileu* hemp seeds treatment

% FA of total FAME†	Colostrum		Milk				*SEM*	*P value‡*
	CL	HSL	CL	HSL	CL	HSL		
	1th AF		7-D AF		21-D AF			
C16:0	19.83	20.12	27.32	33.14	31.49	31.20	*1.316*	**
16-1	3.17	3.28	8.85	11.07	7.59	10.93	*0.784*	***
C17:0	0.49	0.47	0.18	0.21	0.15	0.11	*0.038*	*NS*
C17-1	0.17	0.22	0.20	0.22	0.12	0.15	*0.016*	*NS*
C18:0	4.05	3.88	4.01	4.04	3.66	2.72	*0.129*	*NS*
C18:1n-9 *cis*	29.41	28.26	31.84	22.71	23.97	21.18	*0.861*	**
C18:1n-7 *cis*	1.40	0.65	0.87	0.51	0.49	0.40	*0.161*	*NS*
C18:2n-6 (LA)	34.24	34.95	18.95	18.60	24.34	23.02	*1.689*	***
C20:0	0.23	0.39	0.21	0.23	0.02	0.23	*0.030*	**
C18:3n-3 (ALA)	1.73	2.24	1.20	1.90	1.72	2.52	*0.100*	***
CLA	0.08	0.08	0.06	0.12	0.00	0.09	*0.017*	*NS*
C18:4n-3	0.08	0.01	0.11	0.05	0.14	0.10	*0.170*	*NS*
C20:2n-6	0.09	0.09	0.39	0.11	0.16	0.16	*0.030*	*NS*
C20:3n-6	0.42	0.44	0.14	0.14	-	-	*0.048*	*NS*
C22:1n-9	0.18	0.27	0.04	0.04	-	0.05	*0.029*	*NS*
C20:3n-3	1.00	1.18	0.00	0.07	0.06	0.05	*0.127*	*NS*
C20:4n-6	0.04	0.04	0.71	0.49	0.50	0.45	*0.063*	*NS*
C22:2n-6	0.18	0.20	0.07	0.16	0.05	0.11	*0.026*	NS
C20:5n-3 (EPA)	-	0.02	-	0.01	-	-	*0.006*	NS
C22:4n-6	-	-	-	0.03	0.14	0.16	*0.014*	NS
C22:6n-3 (DHA)	-	-	0.31	0.58	0.15	0.35	*0.054*	NS
∑SFA	27.31	27.54	35.93	42.71	40.20	39.57	*1.511*	*
∑MUFA	34.47	32.89	42.00	34.90	32.51	33.24	*0.788*	*
∑PUFA	37.86	39.29	21.96	22.25	27.25	27.04	*1.781*	NS
∑n-6	35.05	35.82	20.33	19.64	25.18	24.01	*1.661*	NS
∑n-3	2.81	3.46	1.63	2.61	2.06	3.03	*0.162*	**
n-6/n-3	12.66	10.38	12.72	7.56	12.28	8.07	*0.497*	***

†FAME – fatty acids ester methyl; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids;
∑ SFA : C4:0 C6:0+C8:0+ C10:0+ C12:0+ C14:0 + C15:0 + C16:0 + C17:0 + C18:0+ C20:0+ C23:0+C24:0;
∑ MUFA: C14:1+C15:1+ C16:1 + 17:1 + Total cis C18:1 + C20:1 n-9;
∑ PUFA: C18:2n-6 + 18:3n-3 + C18:4 n-3 + CLA + C20:2n-6 + C20:3n-6 +C20:3n-3 + C20:4n-6 + C22:2n-6 + C20:5n-3 +C22:3n-6 + C22:4 n-6 + C22:5 n-3 + C22:6n-3.
‡ NS (P > 0.10), T (P < 0.10), *P < 0.05, **P < 0.01, ***P < 0.001

Source: analyses in Chemistry Lab, INCDBNA, statistic calculation Habeanu

The trend was not similar regarding the time of sampling colostrum/milk for ALA assessment, n-6:n-3 ratio (P>0.05); however, the level of n-3 FA has been influenced significantly by time. The dietary HS induced a significant change of the total SAT and MUFA FA (ΣSFA and ΣMUFA). Predominant among the SFA, was the palmitic acid, whose concentration increased significantly in the colostrum and milk at 7 d and decreased at 21 d in the HS group compared to CL group. The oleic FA (C18:1n-9 *cis)* decreased significantly in HSL group, both in the colostrum and milk.

Discussions

The industrial hemp, with low content of delta-9-tetrahydrocannabinoid (THC, need not to exceed 0.2 %), was accepted to be cultivated in European Union countries [23]. Until 1990, Romania ranked third worldwide in terms of hemp production. Efforts have been made in the recent years to improve the crops and make them suitable for animal feeding. *Jubileu* cultivar, obtained by SCDA Secuieni, Romania, has been approved in 2012 for use as oil and seed crop (900 – 1,200 kg seeds /ha). The quantity and composition of the milk are factors responsible for development of the piglets. The lipid content of diets, as important source of EFA, is essential for obtaining a positive response on health and growth. Results from this study showed that n-3 FA rich diet using HS alter beneficially sow milk composition and litter's performance. The lactating sows treated with HS consumed 8.2% more protein and 16.6% more fat than the control group due to LS that higher as well. It is know that lipid supplementation increases secretion of the milk fat [24], which means a higher level of energy (>1.05 times), protein (> 1.03 times) and fat (>1.02 times) provided to piglets. Furthermore, milk intake of the litter increased gradually during the lactation period and the quantity of nutrient intake depends on MY. Previous studies revealed the relation between the amount of dietary fat and piglet gain [18]. In our study, although the dietary fat level was close, the piglets fed HS, particularly those born by HSL sows, had a stronger development than the piglets born by CL sows and treated with the conventional diet. During the first 7 d after birth, the piglets receive all the required nutrients from the colostrum / milk they suckle, thus the average BW and ADG depending on the treatment applied to the sows. It is generally acknowledged that the heavier piglets and the first to be born have a rapid access to the teats [9] [10] and might thus have a higher intake, which is reflected in their weight gain. From our previous observations, the heavier piglets switch faster to solid food than the lighter piglets.

MY is variable among sows because of multiple factors, such as genetic factors (the MY heritability was estimated at 0.32) [10], nutrition, management, environment [28]. Starting from the model developed by Noblet & Etienne [21], with its limits, and based on the literature, Hansen et al. [12] developed a hierarchical Bayesian model for MY. The data obtained in our trial were included in the regression Eq. described by Hansen et al. [12] Wood [30]. In our study, MY increased up to 19-20 d, when the peak of lactation occurred, then declined; however, a slight increase was noticed in certain sows, MY being correlated with LS and LG. MY enhanced the growth of piglets. For sows with higher MY, LS and LG this finding was close to previous studies [20] [7], were MY appeared to reach the peak between 15-21 d, compared to 19-20 d in our study. When MY was lower, the peak was few days later, which influenced the general mean of peak lactation days. A significantly positive correlation (r=0.93) was noticed between MY and LG, this parameter being included in the Eq. used, which confirms the results of Vadmand et al. [28]. Milk composition, fat particularly, depends on feeding. The rapid changes in the composition of the fluids secreted by the mammary gland influence piglet development. Our results confirm that both MY and its nutrients (protein, fat, lactose) registered a decline starting 20 d AF. In consequence, for further development of the piglets it is better to wean starting with 21 d despite the fact that the enzymatic equipment is underdevelopment. Moreover, as we showed in a previous study that weaning induces an increase of 26%

serum cortisol and 6% Se. By formulating an appropriate diet we could avoid the negative effects of the earlier weaning even more so as after about a week the cortisol value are close to those prior to weaning.

Previous researches [24] [25] showed that little attention has been paid to n-3 FA requirement in sow feeding. Fat is more than energy source, is an EFA source depending on the plants added in the diet. As far as we know, there is no data in the literature regarding the sow milk/colostrum FA composition following HS treatment. In sow colostrum / milk, the chain length is between 4 and 22 carbons. Due to some confusion and regulation, the researches on HS nutritional potential for animals, were not as attractive as another plants known by their EFA rich content and nutritional and health implications. Milk ALA and LA concentration reflected the differences of these FA transferred via sow diet. While colostrum concentration of total n-6 PUFAs of HSL fed group was 2.14% higher than in the CL fed group, the milk level of n-6 PUFAs HSL fed group was lower than in CL group both at 7 d and 21 d. The concentration of n-3 PUFAs was significantly different in colostrum/ milk from HSL sow group. The long chain FA (DHA) increased slowly in HSL group. This registered value led to o significant decrease of n-6:n-3 ratio in the colostrum and milk. This aspect is essential for health. This ratio must be improved to reach the ideal of 5:1 recommended. Higher than 10:1 ratio has adverse health effects. Since similar data on the composition of human milk have been published previously, the results of our study can be the support for further studies on humans and for understanding the correlation with other parameters of reference for the health state.

CONCLUSIONS

The dietary addition of HS influenced in a positive way the milk protein and fat content, due to a higher intake of these nutrients. The sampling day had a significantly higher influence for protein and lactose milk content. The evaluated protein values were slightly higher than the calculated theoretical, whatever the treatment or day, both for fat and lactose the evaluated values were lower. The dietary HS as rich n-3 PUFA vegetable source changed beneficially the FA composition of the sow milk. The quality of the dietary fat had positive effects on animal performance, improving LG and LW. The lactation curve, MY implicitly, changes depending on LG and LS. Significantly positively correlated with MY, milk constituents also changed in time. The results suggested that the HS could be considered a valuable vegetable source due to its beneficial effect on lactating multiparous sows and their litter.

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