# NUTRITIONAL CHARACTERISATION OF HEMP SEEDS AND CAKE AS FUNCTIONAL INGREDIENTS IN RUMINANTS' NUTRITION

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*Our study was aimed to characterise the hemp seeds and cake from the point of view of proximate chemical composition and bioactive compounds, as alternative feedstuffs for ruminants' nutrition. Hemp seeds and cake presented high concentrations of several trace minerals (e.g., iron, manganese, zinc), especially hemp cake had the higher content of these compounds ($0.001<p<0.005$). Also, hemp seeds presented higher concentration of lipophilic antioxidants and essential fatty acids (linoleic and α-linolenic fatty acids) ($p<0.001$). Considering the hydrophilic antioxidant compounds, hemp cake presented higher concentration than hemp seeds ($p<0.001$) and remarkable antioxidant potential, with higher values of the studied antioxidant indices.*

**Keywords**: hemp seeds, hemp cake, antioxidant potential, by-products, ruminants' nutrition

## 1. Introduction

The most important factor that can influence humans' health status is a healthy and balanced diet and this fact can represent a concerning topic for the food industry, considering the rapid growth of the Global population [1]. One of the most important strategies used to naturally enriched animal food products with several nutrients and bioactive compounds is animal nutrition, but the limited resources, water deficiency problem and the massive culture pesticide pollution of the environment, increased the need of valorising several protein sources which have less resources requirements (drought resistant plants, short production cycle or economically valorising of by-products or some industrial wastes) [1]. Hemp *(Cannabis sativa L.)* represents a sustainable alternative to commonly protein sources used in ruminants' nutrition. From the point of view of environment pollution, hemp has a short cropping period, and it needs less water and pesticides

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for development. Moreover, each part of the plant can be economically valorised, without producing wastes [2, 3]. In term of nutritive characteristics, hemp seeds have a nutrients composition comparable with soybeans [4], with considerable amounts of proteins, approximately 25.3% of the dry matter (DM). The main protein component of the hemp seeds is edestin, followed by albumin which is rich in essential amino acids (methionine and cysteine) [5]. Hemp can be used as feedstuff in ruminants' nutrition even after the oily part was removed, obtaining hemp meal or cake, depends on the technology used. After oil extraction, the crude protein composition can be increased approximative at 34.3% DM, making it an excellent protein source for ruminants [5]. Not only the primary chemical composition of hemp makes it an interesting alternative feedstuff in livestock, but also, its composition of bioactive compounds. Hemp represents a rich source of several polyphenols, mainly belong to flavones and flavanols groups, with several beneficial activities for human body (anti-inflammatory, anti-microbial or neuroprotective) [6]. Beside the polyphenols, hemp seeds and cakes have a rich composition of tocopherols, lignans and condensed tannins [7]. In literature are presented few data regarding the hemp influence (seeds or by-products) on milk quality, but in several studies, its utilisation was associated with an increased in milk polyunsaturated fatty acids (PUFA), especially conjugated linoleic acid (CLA), known for its beneficial implication in human health [5, 8]. Moreover, its inclusion in sheep diets led to an increase of the tocopherols content, important from the point of view of oxidative processes that can occur after milk PUFA increase [8].

In this context, the objective of this study was to characterise the hemp seeds and cake, from the point of view of proximate chemical composition and bioactive compounds, as alternative feedstuffs for ruminants' nutrition, with potential beneficial effects on milk quality.

## 2. Materials and Methods

Studied vegetal sources (hemp seeds and cake) were purchased from a local market (Bucharest, Romania).

The crude protein composition of hemp seeds and cake was obtained using Kjeldahl method. Crude fat content was assessed using Soxhlet method and ash content was obtained by heating samples at 550 °C for 24 h using an ashing furnace [9].

Trace minerals concentrations (copper, iron, manganese, zinc) were obtained using flame atomic absorption spectrometry, following the method described by [10]. The calcium concentration was determined according to a complexometric method, by titration of the calcium ions with ethylenediamine tetra-acetic acid (EDTA) solution in the presence of murexide, until the colour turns

purple. The phosphorus content was determined using the reaction with the molybdovanadate reagent, obtaining a yellow-coloured complex that absorbs at 420 nm. The equipment used for phosphorus determination was a Jasco V530 UV/VIS spectrophotometer (Japan Servo Co. Ltd., Japan). Calcium and phosphorus determination were assessed according to Regulation 152/2009 [11].

Antioxidant capacity was performed using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method [12]. The scavenging of samples against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical was performed following the method described by [13] and the total polyphenols content was determined using Folin-Ciocalteu method and total flavonoids content was assessed using the method described by [14]. Total antioxidant capacity (TAC) using the phosphomolybdenum method was performed using the method reported by [12].

Gas chromatography method was used to study the fatty acids profile, using a Perkin Elmer Clarus 500 gas chromatograph (Massachusetts, United States). Fatty acids were esterified in methyl esters, followed by separation on the chromatographic column (60 m x 0.25 mm, 0.25 µm thickness) filled with a high polarity stationary phase (TRACE TR-Fame, Termo Electron, Massachusetts, United States) [15].

Determination of vitamin E isomers (α-, γ-, and δ- tocopherol) concentration was performed using high performance liquid chromatography (HPLC), with Vanquish Core HPLC System (Thermo Fisher Scientific, Bremen, Germany) and an Accucore C18 column (150 x 4.6 mm, 4µm particle size). Mobile phase consisted in 4% water and 96% methanol with a 1 ml/min flow for 20 min. run time. Concentrations of β-carotene and lutein were obtained using HPLC method described by [16].

The profile of polyphenols was obtained using HPLC method with a BDS HyperSil C18 column (250 x 4 mm, 5µm particle size), using for the mobile phase three different solvents (1% acetic acid; methanol and acetonitrile) with a gradient elution program as described by [17].

For each analysis performed in our study, samples were analysed as triplicates (n=3). Statistical analysis was performed by analysis of variance (ANOVA) using Minitab® Statistical Software, version 16. For p values $< 0.05$, the differences among mean values were considered significant. Pearson's correlation coefficient test between the lipophilic compounds (α-tocopherol, γ-tocopherol, δ-tocopherol, total vitamin E, lutein, β-carotene), hydrophilic antioxidant compounds (total polyphenols and total flavonoids) and the parameters that expressed the antioxidant potential of the hemp seeds and cake (DPPH, ABTS, TAC) was presented as correlation heatmap, using Prism-GraphPad software (San Diego, CA, USA). Yellow colour corresponds to positive correlation coefficients and purple colour corresponds to negative correlation coefficients.

## 2. Results and discussions

*Proximate composition of hemp seeds and cake*

The proximate composition of the hemp seeds and cake is presented in table 1. Hemp seeds presented significantly higher composition of crude fat ($p<0.001$) and dry matter ($p = 0.005$), compared with hemp cake. The higher content of crude protein ($p = 0.012$) and crude fibre ($p = 0.001$) was observed in hemp cake. The ash content of the hemp seeds and cake did not significantly differ ($p = 0.205$). The presented data regarding the proximate chemical composition of the studied vegetal sources are in agreement with data presented in literature [18-22], being reported concentrations of crude protein between 20-25%, crude fat 25-35%, ash 4.8-5.6%, dry matter 92.8-98.87%, crude fibre 26.96% for hemp seeds and 24-28% crude protein, 8.7-8.9% crude fat, approximately 30% crude fibre, 5.6% ash and 91.4-92.4% dry matter for hemp cake.

*Table 1*

**The proximate chemical composition of hem seeds and cake (n=3)**

| Proximate chemical composition (%) | Hemp seeds | Hemp cake | SEM | p |
|---|---|---|---|---|
| Crude fat | $31.78^{A}$ | $8.423^{B}$ | 0.409 | <0.001 |
| Crude protein | $23.14^{B}$ | $26.13^{A}$ | 0.480 | 0.012 |
| Crude fibre | $27.70^{B}$ | $34.75^{A}$ | 0.533 | 0.001 |
| Ash | 4.85 | 5.53 | 0.318 | 0.205 |
| Dry matter | $94.53^{A}$ | $90.29^{B}$ | 0.535 | 0.005 |

$^{A, B}$ Means within a row with no common superscript differs ($p < 0.05$)

Considering the data regarding the trace minerals composition of the hemp seeds and cake, presented in table 2, it was observed that hemp cake had the higher composition of cooper, iron, manganese, zinc compared with hempseeds ($0.001<p<0.005$). The higher concentration of calcium was presented in hemp seeds (+24.61% compared with hemp cake). The content of phosphorus did not differ for the studied vegetal sources.

*Table 2*

**Trace minerals composition of hemp seeds and cake (n=3)**

| Trace minerals | Hemp seeds | Hemp cake | SEM | p |
|---|---|---|---|---|
| Cu (mg/kg) | $10.44^{B}$ | $16.44^{A}$ | 0.164 | <0.001 |
| Fe (mg/kg) | $142.70^{B}$ | $151.40^{A}$ | 1.090 | 0.005 |
| Mn (mg/kg) | $89.82^{B}$ | $119.82^{A}$ | 0.880 | <0.001 |
| Zn (mg/kg) | $60.77^{B}$ | $72.85^{A}$ | 0.242 | <0.001 |
| Ca (%) | $0.65^{A}$ | $0.16^{B}$ | 0.005 | <0.001 |
| P (%) | 0.93 | 0.92 | 0.005 | 1 |

$^{A, B}$ Means within a row with no common superscript differs ($p < 0.05$)

Data reported in literature suggested that the main microelement presented in hemp seeds was iron, in a higher content than it was obtained in our study (181-334 mg/kg), followed by manganese (58-81 mg/kg), zinc (55-68 mg/kg), cooper (13-20 mg/kg), calcium (0.10-0.19%) and phosphorus (0.41-0.51%) [23]. Also, in case of hemp cake, the data presented in our study are overall, in agreement with results obtained in other studies. In literature, it was presented a lower concentration of iron (133.6 mg/kg), and phosphorus content (0.71%) and higher content of copper (18.83 mg/kg), manganese (133.0 mg/kg) and zinc (77.83 mg/kg). Calcium content was approximately at the same level as presented in our study (0.17%) [24]. The differences between data obtained in our work and data presented in literature can be explained by the effects of the soil, being well known that minerals are affected directly by the soil properties.

*Bioactive compounds of hemp seeds and cake*

*Lipophilic compounds.* The profile of fatty acids presented in table 3, revealed that the hemp seeds represent a remarkable source of PUFA, Ω3 and Ω6 fatty acids. From the recorded data of fatty acids profile was observed that hemp seeds presented a high content of linoleic and α-linolenic acid. Hemp cake presented higher concentration of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Of these, hemp cake had the higher concentration of short chain fatty acids (SCFA), caproic and lauric acids. The richness of α-linolenic acid for the both hemp seeds and cake led to an excellent ratio between Ω6 and Ω3 fatty acids, according with data presented in literature [25]. The linolenic acid is the most abundant fatty acid in hemp seeds and cake, whose concentration was over 50% in studied vegetal sources, followed by α-linolenic acid whose concentration was above 20%, mainly in hemp seeds, according with data presented in literature [18]. Essential lipidic constituents, such as Ω6 were also presented in high content for the both seeds and cake. Those lipidic compounds are essential for animals, due to their role including the prostaglandin and leukotrienes synthesis.

*Table 3*

**Fatty acids profile of hemp seeds and cake (n=3)**

| g FAME/100g total FAME | | Hemp seeds | Hemp cake | SEM | p |
|---|---|---|---|---|---|
| Caproic Acid | C 6:0 | $0.000^{B}$ | $0.136^{A}$ | 0.014 | 0.002 |
| Lauric Acid | C 12:0 | $0.082^{B}$ | $0.000^{A}$ | 0.006 | 0.001 |
| Myristic Acid | C 14:0 | $0.065^{B}$ | $0.119^{A}$ | 0.009 | 0.012 |
| Myristoleic Acid | C 14:1 | $0.000^{B}$ | $0.015^{A}$ | 0.003 | <0.001 |
| Pentadecanoic Acid | C 15:0 | $0.000^{B}$ | $0.046^{A}$ | 0.002 | <0.001 |
| Pentadecenoic Acid | C 15:1 | $0.011^{B}$ | $0.035^{A}$ | 0.005 | 0.020 |
| Palmitic Acid | C 16:0 | $7.472^{B}$ | $8.432^{A}$ | 0.022 | <0.001 |
| Palmitoleic Acid | C 16:1 | $0.043^{B}$ | $0.178^{A}$ | 0.021 | 0.012 |
| Stearic Acid | C 18:0 | $2.468^{B}$ | $2.802^{A}$ | 0.031 | 0.002 |
| Cis-Oleic Acid | C 18:1 | $11.330^{B}$ | $16.310^{A}$ | 0.035 | <0.001 |
| Cis-Linoleic Acid | C 18:2n6 | $57.680^{A}$ | $53.880^{B}$ | 0.073 | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| Arachidic Acid | C 20:0 | 0.079 | 0.000 | 0.032 | 0.158 |
| α-Linolenic Acid | C 18:3n3 | $19.560^{A}$ | $13.600^{B}$ | 0.039 | <0.001 |
| Heneicosenoic Acid | C 21:0 | $0.000^{B}$ | $0.668^{A}$ | 0.002 | <0.001 |
| Octadecatetraenoic Acid | C18:4n3 | $0.298^{B}$ | $0.782^{A}$ | 0.021 | <0.001 |
| Eicosadienoic Acid | C20(2n6) | $0.319^{A}$ | $0.174^{B}$ | 0.015 | 0.003 |
| Arachidonic Acid | C20(4n6) | 0.079 | 0.000 | 0.032 | 0.158 |
| Other fatty acids | | $0.356^{A}$ | $0.044^{B}$ | 0.019 | <0.001 |
| ΣSFA | | $10.470^{B}$ | $14.830^{A}$ | 0.061 | <0.001 |
| ΣMUFA | | $11.380^{B}$ | $16.540^{A}$ | 0.020 | <0.001 |
| ΣPUFA | | $77.790^{A}$ | $68.580^{B}$ | 0.056 | <0.001 |
| ΣΩ3 | | $19.860^{A}$ | $14.390^{B}$ | 0.019 | <0.001 |
| ΣΩ6 | | $57.940^{A}$ | $54.200^{B}$ | 0.039 | <0.001 |
| Ω6/Ω3 | | $2.918^{B}$ | $3.770^{A}$ | 0.001 | <0.001 |

FAME-fatty acids methyl esters, SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids, Ω3-omega 3 fatty acids, Ω6-omega 6 fatty acids, $^{A, B}$ Means within a row with no common superscript differs ($p < 0.05$)

Moreover, the dietary supplementation of the animals with essential fatty acids sources is important considering that both linoleic acid and α-linolenic are precursors for the biosynthesis of dihomo-γ-linolenic acid, arachidonic acid (precursors of prostaglandins) and respectively, for eicosapentaenoic acid (EPA). From the monounsaturated fatty acids (MUFA) group, oleic acid is one of the most abundant in hemp seeds and cake, being important for ruminant nutrition as literature presented that by enriching the content of oleic acid in milk can be lowered the level of plasma cholesterol, LDL-cholesterol and triacylglycerol concentrations and can reduced the risk for development of the coronary artery disease for milk consumers [25, 26].

Even if the hemp seeds and cake presented high amounts of essential fatty acids constituents, it must be taken into consideration the oxidative effects of unsaturated fatty acids which can be slowed by natural antioxidants. Therefore, the lipophilic antioxidant compounds (table 4) were studied to evaluate the antioxidant potential of the studied vegetal sources. Hemp seeds presented higher concentration of major lipophilic antioxidants, compared with hemp cake. Also, it presented an excellent concentration of vitamin E, our results being according with data presented in other studies [27]. The major isomer of vitamin E found in hemp seeds and cake was γ-tocopherol [28], which exert the most powerful influence in free radical removing, among the vitamin E isomers, and also can be related to carcinogenesis inhibition [29]. The main carotenoids presented in hemp seeds and cake were lutein and β-carotene.

*Table 4*

**Lipophilic antioxidant compounds of hemp seeds and cake (n=3)**

| Lipophilic compounds (mg/kg) | Hemp seeds | Hemp cake | SEM | p |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| α-tocopherol | 20.050$^{A}$ | 2.935$^{B}$ | 0.087 | <0.001 |
| γ-tocopherol | 156.930$^{A}$ | 21.770$^{B}$ | 0.823 | <0.001 |
| δ-tocopherol | 24.720$^{A}$ | 5.590$^{B}$ | 0.131 | <0.001 |
| Total vitamin E | 201.690$^{A}$ | 30.300$^{B}$ | 0.776 | <0.001 |
| Lutein | 63.330$^{A}$ | 40.070$^{B}$ | 1.091 | <0.001 |
| β-carotene | 39.090$^{A}$ | 9.810$^{B}$ | 1.260 | <0.001 |

$^{A, B}$ Means within a row with no common superscript differs ($p < 0.05$)

The data obtained for lutein concentration were higher than several data reported in literature (33.9 mg/kg and 40.4 mg/kg in whole and defatted hemp seeds) [30]. Also, the β-carotene was not detected by other authors in whole seeds, but was presented in hemp cake in amount of 0.017 mg/g oil, lower concentration than data obtained in our study [30]. The same study reported a lower concentration of β-carotene in whole and defatted hemp seeds than data obtained in our work (approximately 0.4-0.6 µg/g) [30]. The lower concentration of the major lipophilic compounds found in hemp cake compared with the whole hemp seeds obtained in our study can be related to the oil removal technology. After the oil removing a part of the bioactive compounds found in oily part can remain in the pressed cakes and can exert biological activity [31].

*Hydrophilic compounds.* In case of hydrophilic antioxidants (table 5), hemp cake presented the richest composition of the major polyphenols detected in studied vegetal sources. The main polyphenol class presented in higher concentration both in hemp seeds and cake was the polyphenolic acids group. The major polyphenol detected was caffeic acid, followed by chlorogenic acid, syringic acid and gallic acid. Although, in case of hemp cake was observed that the individual and total content of polyphenols were significantly higher than in seeds, explained by the oil extraction process. Other studies reported that oil seed cakes presented the richest quantity of polyphenols than the corresponding whole seeds. Also, after oily part removing, the polyphenols of the cake can be more easily extracted, being more available in the matrix [32, 33].

*Table 5*

**Polyphenols profiles of hemp seeds and cake (n=3)**

| Polyphenols (mg/g) | Hemp seeds | Hemp cake | SEM | p |
|---|---|---|---|---|
| Phenolic acids | | | | |
| Gallic Acid | 0.009$^{B}$ | 0.046$^{A}$ | 0.001 | <0.001 |
| Syringic acid | 0.018$^{B}$ | 0.055$^{A}$ | 0.001 | <0.001 |
| Protocatechuic acid | 0.015$^{A}$ | 0.009$^{B}$ | 0.0004 | 0.001 |
| Chlorogenic acid | 0.000$^{B}$ | 0.055$^{A}$ | 0.0006 | <0.001 |
| Methoxy cinnamic acid | 0.008$^{B}$ | 0.015$^{A}$ | 0.0003 | <0.001 |
| Caffeic acid | 0.063$^{B}$ | 0.150$^{A}$ | 0.001 | <0.001 |
| Flavonoids | | | | |

| | | | | |
|---|---|---|---|---|
| Catechin | $0.000^{B}$ | $0.051^{A}$ | 0.0008 | <0.001 |
| Rutin | $0.007^{B}$ | $0.009^{A}$ | 0.0002 | 0.002 |
| Stilbene | | | | |
| Resveratrol | $0.010^{A}$ | $0.008^{B}$ | 0.0002 | 0.002 |
| Total | $0.132^{B}$ | $0.401^{A}$ | 0.003 | <0.001 |

$^{A, B}$ Means within a row with no common superscript differs ($p < 0.05$)

In literature are few data regarding the profile of polyphenols in both hemp seeds and cake. The data regarding the polyphenols profile of hemp seeds and cake are contradictory, few studies reported that the major polyphenols group found in hemp seeds is represented by flavonoids class [34], while other authors [35] reported that the main polyphenol presented in hemp cake is quercetin, followed by luteolin and caffeic acid, with the lowest concentration, in contradiction with our study.

*Antioxidant potential of hemp seeds and cake*

Considering the high concentration of antioxidants in hemp seeds and cake, the antioxidant potential of the studied vegetal sources was assessed, and the results are presented in table 6.

*Table 6*

**Antioxidant potential of hemp seeds and cake (n=3)**

| Antioxidant potential parameters | Hemp seeds | Hemp cake | SEM | p |
|---|---|---|---|---|
| Total polyphenols (mg/g GAE) | $0.466^{B}$ | $2.874^{A}$ | 0.107 | <0.001 |
| Total flavonoids (mg/g QE) | $0.000^{B}$ | $0.431^{A}$ | 0.007 | <0.001 |
| DPPH (mM Eq. Trolox) | $39.990^{B}$ | $146.900^{A}$ | 8.636 | <0.001 |
| ABTS (mM Trolox) | $4.055^{B}$ | $32.212^{A}$ | 2.739 | 0.002 |
| TAC (mM Eq. Ascorbic acid) | $38.540^{B}$ | $58.970^{A}$ | 0.612 | <0.001 |

GAE-gallic acid equivalents, QE-quercetin equivalents, DPPH-antioxidant capacity using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate method, ABTS- scavenging of samples against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) EDTA-ethylenediaminetetraacetic acid equivalents, TAC-total antioxidant capacity using phosphomolybdenum metho, $^{A, B}$ Means within a row with no common superscript differs ($p < 0.05$).

Hemp cake possessed the highest antioxidant potential, with highest total polyphenols content, total flavonoids, DPPH, ABTS and TAC, comparing with hemp seeds. These results can be explained by the evidence regarding the biological activity of phenolic compounds in hemp seeds and cake. After extraction of seeds oil, the polyphenols, that usually are conjugated with macronutrients are released and their bioavailability can be improved [36].

In Fig. 1 can be found the Pearson's correlation coefficient test, presented as correlation heatmap between the lipophilic compounds (α-tocopherol, γ-tocopherol, δ-tocopherol, total vitamin E, lutein and β-carotene), hydrophilic

antioxidant compounds (total polyphenols and total flavonoids) and the parameters that expressed the antioxidant potential of the hemp seeds and cake (DPPH, ABTS TAC).

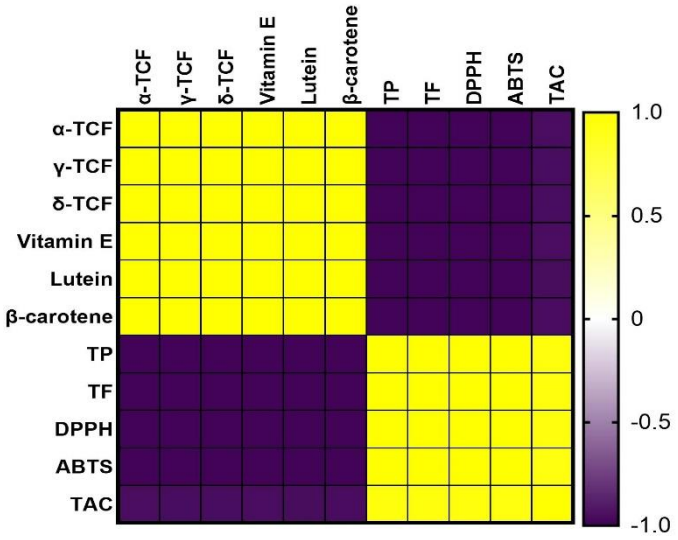


Fig. 1. Heat map presenting the Pearson's correlations between the lipophilic (α-tocopherol, γ-tocopherol, δ-tocopherol, total vitamin E, lutein, β-carotene), hydrophilic antioxidant compounds (total polyphenols and total flavonoids) and the parameters that expressed the antioxidant potential of the hemp seeds and cake (DPPH, ABTS, TAC). Abbreviations: α-TCF=α-tocopherol, γ-TCF=γ-tocopherol, δ-TCF=δ-tocopherol, TP=total polyphenols, TF=total flavonoids.

It was revealed that the lipophilic antioxidant compounds are positively correlated with each other (r=1.00). The hydrophilic antioxidant compounds were strongly positive correlated with DPPH, ABTS and phosphomolybdic antioxidant capacity. Negative correlations were presented between the parameters that expressed the antioxidant potential (DPPH, ABTS and phosphomolybdic antioxidant capacity) and lipophilic antioxidants.

## 4. Conclusions

Our study revealed that both hemp seeds and cake can be a rich source of bioactive compounds. Hemp seeds presented excellent composition of lipophilic compounds such as essential fatty acids (linoleic acid and α-linolenic fatty acids), Ω3 and Ω6 fatty acids, vitamin E and carotenoids. Otherwise, hemp cake presented higher concentration of hydrophilic antioxidants (polyphenols) and exerted higher antioxidant activity, due to its rich polyphenols content. Both hemp seeds and cake

can represent important alternative feedstuffs for ruminants due to their composition rich in macro- and micro- nutrients, leading to naturally enriched dairy products. Should be noted that the highest unsaturated fatty acids composition presented in hemp seeds can led to an increased risk of lipid oxidation and must be administrate with prevention, or in combination with several antioxidants. In contrast, hemp cake represented a cheapest choice comparing with corresponding seeds, with lower concentration of unsaturated fatty acids, but higher concentration of polyphenols and powerful antioxidant capacity.

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