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SHORT COMMUNICATION
Effects of hemp leaf, hemp seed, and pelleted hempseed meal on *in vitro* rumen fermentation and methane production

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Abstract. This study evaluated the effects of hemp leaf (HL), hemp seed (HS), and pelleted hempseed meal (HP) on rumen fermentation parameters, and feed value using the *in vitro* gas production technique. Chemical analyses showed highly significant differences between the three hemp-derived feed materials ($P < 0.01$). Except for microbial protein (MP; $P > 0.05$), all evaluated *in vitro* fermentation parameters, including gas production (GP), methane production (ml and %), truly digested dry matter (TDDM), partitioning factor (PF), and microbial protein synthesis efficiency (MPSE) differed significantly between the groups ($P < 0.01$). Feed value was assessed based on organic matter digestibility (OMD), neutral detergent fibre digestibility (NDFD), metabolizable energy (ME), net energy for lactation (NEL), dry matter digestibility (DMD), dry matter intake (DMI), relative feed value (RFV), and relative forage quality (RFQ). Hemp seed and hempseed pelleted meal showed similar values for most evaluated

parameters, differing only in OMD, whereas hemp leaf had the highest values for most fermentation and feed quality parameters. Differences among the groups were significant for all feed value indices ($P < 0.01$). In conclusion, HL, HS, and HP show potential as alternative energy and protein sources in ruminant diets. However, further *in vivo* studies are needed to confirm their effects on animal performance and health.
Keywords: Digestibility, hemp, methane emission, nutrient composition

Introduction

Hemp (*Cannabis sativa* L.) is considered a promising feed resource for ruminants because of its high nutritional value and adaptability (Gurbuz, et.al., 2022). The global livestock industry faces several challenges, including meeting the increasing protein demand of a growing population while ensuring environmental sustainability, minimising feed costs, and maintaining animal welfare (Bailoni et al., 2021; Altman et al., 2023). Modern industrial hemp cultivars contain less than 0.3% tetrahydrocannabinol (THC), limiting their psychoactive potential (Small and Cronquist, 1976). Following their use in the fibre and oil industries, hemp by-products, including leaves, stems, seeds, and hempseed meal, can be used as animal feed because of their high nutritional value. Hemp leaf (HL), hemp seed (HS), and pelleted hempseed meal (HP) are valuable sources of protein, lipids, essential fatty acids, particularly omega-3 and omega-6 fatty acids, antioxidants such as flavonoids and terpenes, vitamins, including vitamins E, D, and A, and fibre fractions such as cellulose and hemicellulose (Rapetti et al., 2021; Vastolo et al., 2021; Wang et al., 2022; Kwon et al., 2023; Frazzini et al., 2024; Rosso et al., 2024). There is also evidence indicating that hemp and its by-products may improve milk and meat quality. Their inclusion in ruminant nutrition has been reported to increase the concentrations of conjugated linoleic acid (CLA) and omega-3 fatty acids in milk and dairy products (Mierliță, 2018; Bailoni et al., 2021; Papatzimos and Kasapidou, 2024). Several studies have shown that hemp has high antioxidant capacity, which may improve oxidative stability and extend the shelf life of meat (Semwogerere et al., 2020; Iommelli et al., 2024). Hemp by-products may also increase the amount of bypass protein reaching the small intestine because of their protein characteristics (Gurbuz et.al 2022). In addition, polyphenols and condensed tannins present in hemp may contribute to controlling certain gastrointestinal parasites and improving intestinal health. The antimicrobial properties of cannabidiol (CBD) have recently been reviewed, suggesting its potential as a new class of antibacterial agents. CBD has shown antibacterial activity against highly resistant bacterial species, including *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Clostridioides difficile* (Lanzoni et al., 2023; Semwogerere et al., 2020; Bailoni et al., 2021).

Hemp plants and their derivatives may also improve digestibility, reduce methane emissions, and support rumen health. *In vitro* studies have shown that supplementation with hemp leaf extract at 6% dry matter (DM) reduces total gas and methane production while optimising nutrient digestibility and rumen pH. Hemp leaf extract has also been reported to promote the growth of cellulolytic bacteria such as *Ruminococcus albus* and *R. flavefaciens*. In addition, it may increase ammonia nitrogen concentration and the proportion of propionate in the volatile fatty acid (VFA) profile (Stevens et.al., 2022; Lambo et al., 2024). These changes indicate that hemp may positively affect rumen health and reduce methane generation (Phupaboon et al., 2024). Similarly, polyphenols and other phytochemicals present in hemp may influence the rumen microbiota and reduce methane production, thereby lowering energy losses associated with methanogenesis (Vasta et al., 2019; Khejornsart et al., 2021; Lambo et al., 2024; Irawan et al., 2024). Because hemp contains cannabinoids, some countries have established legal limits for THC and its derivatives in animal products (Rocca and Salvo, 2020; Altman et al., 2023; Yano and Fu, 2023). In the industrial hemp sector, residual by-products generated during processing generally contain lower levels of anti-nutritional factors. In addition, physical, chemical, or enzymatic treatments can be applied to reduce or remove potentially undesirable compounds from feed materials (Miranda-Cortés et al., 2023; Irawan et al., 2024; Stevens et al., 2022). Overall, hemp and its by-products have potential as sustainable and functional alternative feed resources in ruminant nutrition. However, further studies are needed to determine appropriate inclusion levels and practical feeding strategies.

Materials and Methods

Material

Hemp leaves and seeds used in this study were obtained from experimental plots at the Hemp Research Institute of Yozgat Bozok University (Yozgat, Türkiye). Hemp leaves were harvested at the end of the flowering period. Leaves were collected according to the AOAC (1998) sampling protocol by sampling the field in a zigzag (“W”) pattern. Samples were collected from 10–15 representative locations, and leaves from approximately 20% of the plants were pooled to obtain a composite sample of 60–80 leaves. Seed samples were collected from the same experimental plots.

The seeds were cleaned, dried to a moisture content below 10%, and subjected to hot pressing (40–50 °C) to extract the oil. The resulting hempseed meal was ground, conditioned with steam to a moisture content of approximately 16% at 80–90 °C, pelleted using a 4 mm die, cut into approx.1 cm fragments, dried to a final moisture content of 12%, cooled, and packaged (Gürbüz and Arslan 2017). Before chemical analyses and *in vitro* incubation, HL, HS, and HP samples were ground through a 0.5 mm sieve to ensure a uniform particle size (NRC, 2001). The *in vitro* experiments were conducted at the Atatürk University Animal Nutrition Laboratory. Rumen fluid was collected from three two-year-old Brown Swiss cattle immediately after slaughter at the Erzurum Meat and Milk Institution. Approximately 500 ml of rumen fluid was collected within 5 min after opening the rumen, transferred into CO_2-flushed thermos flasks maintained at approximately 39.5 °C, and transported immediately to the laboratory for *in vitro* analyses under anaerobic conditions (Fortina et al., 2022).

In vitro experiment

After establishing the experimental groups samples of hemp leaf, hemp seed, and pelleted hempseed meal were analysed in triplicate DM, crude ash (CA), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to AOAC (1998). NDF, ADF, and ADL were analysed using the Ankom[200] Fiber Analyzer (Ankom Technology) to determine NDF and ADF following the procedure of Mertens (2003) for NDF and Van Soest et al. (1991) for ADF (AOAC, 1995). The chemical composition of the experimental feed materials is presented in Table 1. Relative feed value (RFV) was calculated according to Van Dyke and Anderson (2000) using the following equations:

$$\text{DMD (\%)} = 88.9 - 0.779 \times \text{ADF (\%)}$$

$$\text{DMI (\% body weight)} = 120 / \text{NDF (\%)}$$

$$\text{RFV} = \text{DMD} \times \text{DMI} / 1.29.$$

In vitro digestibility was determined using the ANKOM Daisy Incubator method. Hemp leaf, hemp seed, and pelleted hempseed meal samples were incubated for 48 h to determine true dry matter digestibility (TDDM), true NDF digestibility (TNDFD), dry matter intake (DMI), and total digestible nutrients (TDN). Relative forage quality (RFQ) was calculated according to Ward and Ondarza (2008) using the following equation:

$$\text{RFQ} = \text{DMI (\% DM)} \times \text{TDN (\% DM)} / 1.23$$

organic matter digestibility (OMD), ME, and net energy for lactation (NEL) were calculated using the equations provided by Menke and Steingass (1988):

$$\text{OMD (\%)} = 15.38 + 0.8453 \times \text{GP} + 0.0595 \times \text{CP} + 0.0675 \times \text{CA}$$

$$\text{ME (MJ/kg DM)} = 2.20 + 0.1357 \times \text{GP} + 0.057 \times \text{CP} + 0.002859 \times \text{EE}^2$$

$$\text{NEL (MJ/kg DM)} = 0.101 \times \text{GP} + 0.051 \times \text{CP} + 0.112 \times \text{EE}$$

where GP represents the net gas production after 24 h.

For the gas production assay, 500 mg of each sample was placed into 100 ml glass syringes maintained in a water bath at 39.5 °C according to Menke et al. (1979). Buffered rumen fluid was added, and the syringes were incubated for 24 h. Gas and methane production were measured using a Sensor Europe Methane Analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). After gas measurements, the fermentation residues were treated with NDF solution for 1 h, and the analysis was continued according to the procedure described by Van Soest et al. (1991).

TDDM, TD, microbial protein synthesis efficiency (MPSE), MP, and partitioning factor (PF) were calculated according to Blümmel et al. (1997) using the following equations:

TDDM (mg) = Incubated DM (mg) – Residual DM (mg)

TD (%) = [(Incubated DM – Residual DM) / Incubated DM] × 100

MPSE = [TDDM – GP × 2.2 mg/ml] / TDDM

MP (mg/g DM) = TDDM – GP × 2.2 mg/ml

PF = TDDM / GP

The data were analysed using SPSS version 27. One-way analysis of variance (ANOVA) was performed, and differences between groups were assessed using Duncan's multiple range test (MacInnes, 2016).

Results and Discussion

Chemical composition of hemp and its by-products

According to the chemical analysis (Table 1), DM differed significantly between the experimental groups ($P < 0.05$), while highly significant differences were determined for all other chemical composition parameters ($P < 0.01$). HS and HP meal showed similar DM contents, whereas the HL group differed significantly from the other groups ($P < 0.05$). HL differed from both groups. HS had the lowest CA content but the highest EE, NDF, ADF, and ADL contents. CP also differed significantly among the feed materials, with the highest value observed in HP (approximately 40%).

The chemical composition of hemp fractions varies considerably and determines their potential use in animal nutrition. The present results agree with those reported by Kleinhenz et al. (2020), who reported that hemp seeds and hempseed meal are rich sources of protein and energy, while hemp leaves contain higher fibre concentrations. Similarly, Jacobson et al. (2021) reported mean values of 50.1 NDF, 36.1 ADF, 12.9 ADL, and 30.9% CP in hempseed meal, which are generally consistent with the present findings. Moreover, comparable observations were reported by Galasso et al. (2016), Kleinhenz et al. (2020), and Fruge et al. (2024), who suggested that hemp seed and hempseed meal can serve as protein and energy sources, whereas hemp leaves are better suited as a fibre source. Likewise, Vastolo et al. (2021) observed lower concentrations of CP (20.23%), ether extract (EE) (6.71%), CA (6.98%), NDF (31.29%), ADF (17.02%), and ADL (6.85%) in hemp inflorescence, oil, and meal, although the overall compositional pattern was similar. Overall, the nutrient compositions of hemp leaves, seeds, and meal vary markedly between different plant parts, cultivars, and growing conditions, indicating their suitability as alternative feed resources with different nutritional applications.

Effects of hemp and its by-products on *in vitro* parameters

The *in vitro* parameters of HL, HS, and HP are presented in Table 2. Except for microbial protein (MP) ($P > 0.05$), all evaluated parameters differed significantly between the three feed materials ($P < 0.01$).

TDDM differed markedly among the treatments, with the highest value observed for HL (416.119 mg), followed by HS (293.668 mg) and HP (269.244 mg). These results are consistent with the findings of Fruge et al. (2024), who reported high *in vitro* digestibility of hemp leaves. Similarly, Jacobson et al. (2021) observed that dry matter digestibility decreased with increasing dietary protein content, which is in line with the present findings, as the feed material with the highest CP content (pelleted HP) showed the lowest TDDM.

Figure 1. shows clear differences in TDDM, PF, MP, and MPSE among the three hemp feed materials. HL showed the greatest degradability, whereas HS had the highest PF and MPSE

values. Although MP values were numerically higher in HS, differences between groups were not significant ($P = 0.055$). These findings suggest that hemp leaf is more degradable during rumen fermentation, whereas hemp seed may provide more efficient microbial protein synthesis. In another study conducted on rumen microorganisms, Denisenko et al. (2025) showed that incrementing proportion of hemp meal (14, 20, and 26%) resulted in increased *in vitro* digestibility by 1.5, 3.81, and 4.82%, respectively, compared to the barley control diet. They also observed increased protozoal counts with raising hemp inclusion, which could favour methanogen activity and increase methane production. The present results are generally consistent with these observations. Likewise, Phupaboon et al. (2024) reported that dietary inclusion of HL at levels up to 6% favourably affected methane and cumulative gas production, supporting the potential utilisation of this material in ruminant nutrition.

Figure 2 shows that HL had the highest GP (112.983 ml) and methane volume (18.793 ml), followed by HP (44.017 and 9.703 ml, respectively), whereas HS reached the lowest values (37.457 and 6.637 ml, respectively). Differences between the feed materials were significant for both parameters ($P < 0.001$).

Similarly, Vastolo et al. (2021) reported methane production of 10.39% after 24 h of incubation of hempseed meal, which is comparable to the values obtained in the present study. The lower methane production recorded for HP may be related to its fatty acid content, particularly essential fatty acids, which may inhibit the development of methanogenic microorganisms in the rumen. Differences among studies may also reflect variation in hemp genotype, plant fraction, processing method, and rumen microbial composition (Kleinhenz et al., 2020). Similarly, the plant part used, such as cotyledon, apical meristem, or leaf segment, as well as the physiological status of the plant material evaluated in the studies, may have a substantial influence on the results. Consequently, the major challenge in developing a standardised and reproducible protocol is the need to optimise these multifactorial interactions separately for each hemp genotype and rumen microbial population.

Effects of hemp and its by-products on feed quality parameters

The present findings showed that HS and HP had similar values for all evaluated parameters except OMD (Table 3). HL reached the highest values for most parameters. Except for NDFD ($P > 0.05$), all feed quality parameters differed significantly between the three feed materials ($P < 0.01$).

Hemp and its by-products are characterised by high protein, fibre, and fat contents, and are rich in rumen bypass protein, essential amino acids, and polyunsaturated fatty acids (Serrapica et al., 2019; Bailoni et al., 2021; Kasula et al., 2021; Winders et al., 2023). The current results are consistent with those reported by Wang et al. (2022), who reported ME and NEL values of 6.77 MJ/kg M and 3.26 MJ/kg DM, respectively, for different industrial hemp products, including seeds, pelleted seed meal, seed meal, seed oil, protein concentrate, and silage. Also, Serrapica et al. (2019) reported ME value of 8.1 MJ/kg DM for pelleted hempseed meal. In addition, Winders et al. (2023) also observed that dietary inclusion of hempseed meal improved rumen fermentation and digestibility in cattle.

Figure 3 shows that HL had the highest values for all percentage-based nutritional parameters. OMD, dry matter digestibility (DMD), DMI, RFV, and RFQ were significantly higher in HL than in HS and HP **(**$P < 0.001$), indicating better forage quality and feeding value. Meanwhile, HP had a higher OMD than HS but the lowest DMD and RFV values. DMI and RFQ were similar between HS and HP. Although HL also had the highest NDFD, the differences between feed materials were not statistically significant ($P = 0.098$). Overall, HL appears to have better digestibility, predicted intake potential, and forage quality than HS and HP.

These results are consistent with the data obtained in the present study. Higher RFV and RFQ indicate greater feed intake potential and digestibility, and may positively affect animal performance. Therefore, feeds with high RFV and RFQ values are preferred in ration formulation. Here, the higher DMD and DMI values of HL contributed to its higher RFV and RFQ. This result can be explained by the high digestibility of the leafy and floral parts of hemp, which, according to reports, reaches 93% for leaves and 87% for flowers (Gurbuz et al. 2022), indicating that these plant parts can be a valuable source of feed for ruminants. In contrast, HS and its by-products, including meal, and pellets, are rich in rumen-undegradable protein, essential amino acids, and unsaturated fatty acids. Therefore, rather than improving feed quality parameters such as OMD, ME, NDFD, NEL, DMD, and DMI, they may contribute more strongly to animal production by improving the nutritional quality of animal products and overall performance. Previous studies have also demonstrated that hemp-derived feed ingredients may improve the fatty acid profile of milk and meat through their content of omega-3 and omega-6 fatty acids and conjugated linoleic acid (Della Rocca and Di Salvo, 2020; Bailoni et al., 2021; Wang et al., 2022; Lanzoni et al., 2023; Yano and Fu, 2023; Fruge et al., 2024; Podder et al., 2024). Different hemp fractions showed distinct nutritional characteristics in the current work. HP contained the highest CP (40.11%), while HS had the highest crude fat content (18.28%). In contrast, HL, despite having a lower protein content, reached higher digestibility values, which may be associated with its lower NDF, ADF, and ADL contents. These findings are consistent with those of Jacobson et al. (2021), Kleinhenz et al. (2020) and Wang et al. (2022).

When the *in vitro* fermentation results were evaluated, HL showed the highest GP, TDDM, OMD, ME, NEL, RFV, and RFQ values. In contrast, digestibility was lower in the HS and HP groups. The decrease in TDDM with increasing CP content is in line with the findings of Jacobson et al. (2021). Although HL produced the highest GP, the proportion of methane in total gas was higher in HP. This difference may reflect variation in the fermentation characteristics of the different hemp fractions. Overall, the present findings are consistent with previous studies showing that hemp and its by-products can influence rumen fermentation and methane production (Vastolo et al., 2021; Phupaboon et al., 2024).

Taken together, the findings indicate that hemp leaf is a high-quality roughage source due to its high digestibility and favourable feed quality parameters. In contrast, HS, and especially HP, can be used as an alternative concentrate feed owing to its high protein and energy content. Therefore different hemp fractions should be used in a complementary manner according to the nutritional objectives and requirements of the ration.

Conclusion

Hemp leaves, hemp seeds, and pelleted hempseed meal have potential as alternative feed resources for livestock. Hemp leaves were characterized by higher digestibility and feed quality parameters, whereas hemp seed and pelleted hempseed meal provided higher protein and energy contents. These findings indicate that different hemp fractions may play complementary roles in ruminant nutrition. hemp fractions differed considerably in nutrient composition, digestibility, and methane production. Hemp leaf showed the highest digestibility, feed value, and energy potential, but also produced the greatest amount of methane. In contrast, hemp seed generated the lowest total gas and methane volumes, indicating the most favourable potential for methane mitigation, while also providing a high ether extract content. Pelleted hempseed meal produced less methane than hemp leaf and, with approximately 40% crude protein, represented a promising alternative protein source, although its digestibility was lower. Overall, hemp seed and pelleted hempseed meal may offer nutritional value with comparatively lower methane emissions; however, these effects should be confirmed through further *in vivo* studies.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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Table 1. Chemical composition of hemp leaf, hemp seed, and pelleted hempseed meal

Feed material, %	DM	CA	CP	EE	NDF	ADF	ADL
Hemp leaf (HL)	91.147^{a}	17.160^{c}	14.587^{a}	2.753^{a}	35.103^{a}	13.033^{a}	5.397^{a}
Hemp seed (HS)	94.473^{b}	4.380^{a}	25.287^{b}	18.283^{b}	60.730^{c}	44.333^{c}	32.947^{c}
Hempseed pelleted meal (HP)	94.413^{b}	9.237^{b}	40.113^{c}	1.623^{a}	57.997^{b}	40.240^{b}	29.470^{b}
P-value	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

DM – dry matter, CA – crude ash, CP – crude protein, EE – ether extract, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin;
^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 2. *In vitro* parameter values of hemp leaf, hemp seed, and hempseed pelleted meal

Feed material	GP ml	Methane ml	Methane %	TDDM mg	PF mg	MP mg	MPSE mg
Hemp leaf (HL)	112.983^{c}	18.793^{c}	16.613^{a}	416.119^{b}	4.436^{a}	209.539^{ab}	50.351^{a}
Hemp seed (HS)	37.457^{a}	6.637^{a}	17.673^{a}	293.668^{a}	9.431^{c}	225.181^{b}	76.658^{c}
Hempseed pelleted meal (HP)	44.017^{b}	9.703^{b}	22.033^{b}	269.244^{a}	7.362^{b}	188.767^{a}	70.102^{b}
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.055	< 0.001

GP – gas production, TDDM – truly digestible dry matter, PF – partitioning factor, MP – microbial protein, MPSE – microbial protein synthesis efficiency;
^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 3. Effects of hemp leaf, hemp seed, and pelleted hempseed pelleted meal on relative feed value, relative forage quality, and other parameters

Feed material	OMD %	NDFD %	ME, MJ/kg DM	NEL, MJ/kg DM	DMD %	DMI %	RFV %	RFQ %
Hemp leaf (HL)	94.230^{c}	34.070^{a}	9.187^{b}	5.440^{b}	47.443^{b}	3.421^{b}	125.756^{b}	228.506^{b}
Hemp seed (HS)	76.880^{a}	28.863^{a}	6.553^{a}	3.477^{a}	28.730^{a}	1.976^{a}	43.972^{a}	92.485^{a}
Pelleted hempseed meal	89.323^{b}	29.477^{a}	6.883^{a}	3.757^{a}	23.660^{a}	2.069^{a}	37.934^{a}	89.386^{a}
P-value	< 0.001	0.098	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note: Means with different superscript letters within the same column differ significantly ($P < 0.05$). OMD – organic matter digestibility, NDFD – neutral detergent fiber digestibility, ME – metabolizable energy, NEL – net energy for lactation, DMD – dry matter digestibility, DMI – dry matter intake, RFV – relative feed value, RFQ – relative forage quality
^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

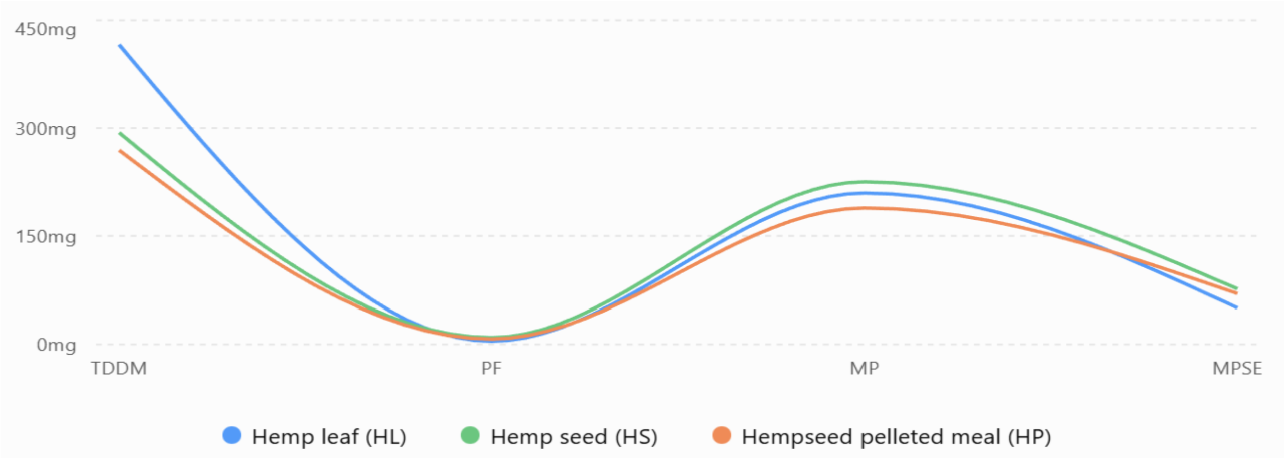

Figure 1. TDDM, PF, MP, and MPSE values *in vitro* parameters of hemp leaf, hemp seed, and hempseed pelleted meal

*TDDM – truly digestible dry matter, PF – partitioning factor, MP – microbial protein, MPSE – microbial protein synthesis efficiency

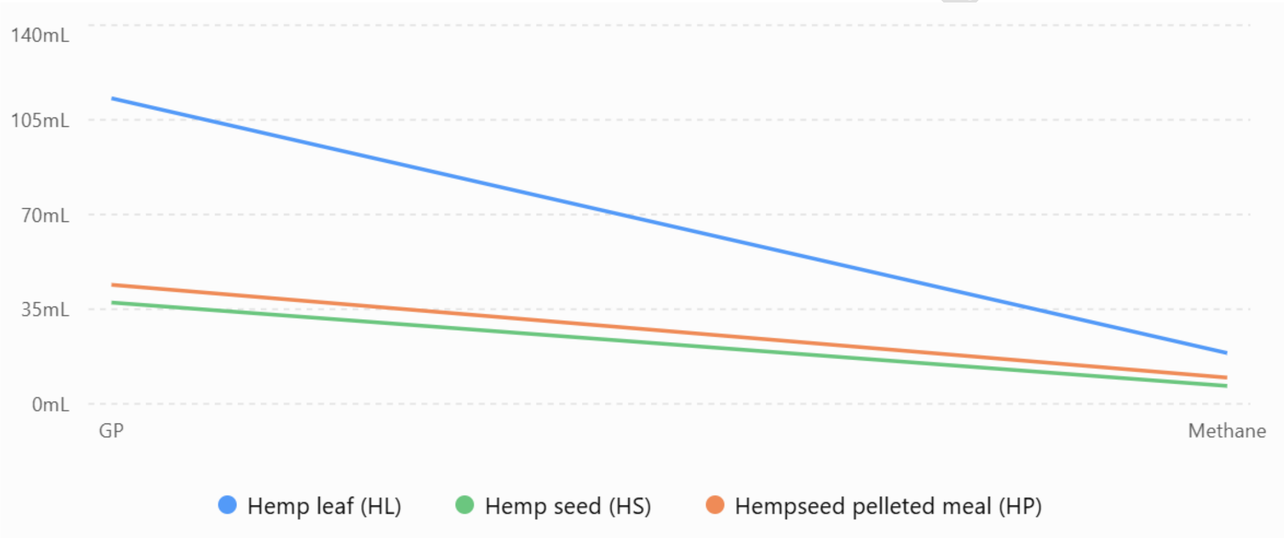

Figure 2. Gas production and methane gas production values of hemp leaf, hemp seed and hempseed pelleted meal

- GP – gas production, Methane – Methane gas amount

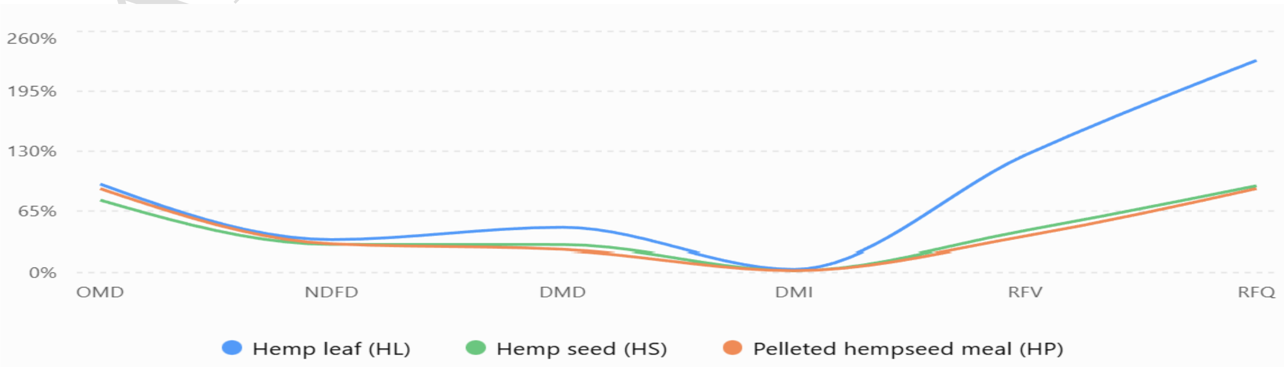

Figure 3. Percentage-based nutritional parameters of hemp feed materials

*OMD – organic matter digestibility, NDFD – neutral detergent fiber digestibility, DMD – dry matter digestibility, DMI – dry matter intake, RFV – relative feed value, RFQ – relative forage quality