

Feeding Spent Hemp Biomass to Dairy Cattle

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Abstract

In the US, hemp containing $\leq 0.3\%$ tetrahydrocannabinol (THC) is now legal to cultivate. In the US, $>60\%$ of hemp is cultivated for cannabidiol (CBD) extraction, producing a large amount of spent hemp biomass (SHB) that have little or no use. The biomass could be used as feed for dairy cows, but it has not yet received approval by the FDA-CVM. The analysis of the SHB revealed a high nutritive quality that is at par or even better than alfalfa. The nutritional composition among hemp batches is quite consistent, except for NDF (23 to 44%) and fat (3 to 7.5%), as consequence of different extraction methods. As sequence, the energy level of SHB is variable (1.12 to 1.59 Mcal/kg). The SHB has a high content of both macro- and micro-minerals that can benefit dairy cows. The amino acid profiling of SHB, except for a lower Lys, is appropriate for dairy cows, and the fatty acid profiling is very similar to alfalfa with high omega-3 content. The SHB contains around 1.6% cannabinoids (from 0.5 to 3%), with CBD and CBDA being the most abundant (0.52 and 0.86%, respectively) and with traces of THC (from no detectable to 0.07%). No pesticides and mycotoxins were detected and amounts of heavy metals are very low. The presence of cannabinoids in SHB, especially CBD, can positively impact the health of dairy cows, especially during the peripartum due to the positive effects observed

in monogastric animals on the immune system, including anti-inflammatory and antioxidant effects. Presence of cannabinoids and other secondary compounds indicate the possible use of SHB as a nutraceutical. In our initial study in finishing rams, feeding up to 20% SHB did not affect health or performance of the animals. The presence of cannabinoids in SHB is the major reason for the legal aspect related to the use of SHB with livestock. No data are available yet on the transfer of cannabinoids in milk and meat in animals fed with SHB, and in vivo studies to assess the residuals of cannabinoids in milk and meat after feeding SHB are warranted. Estimates indicate levels of cannabinoids in milk and meat that should not affect human health.

Introduction

Hemp is a crop that belongs to the Cannabaceae family. Hemp, with more than 25,000 identified uses, is one of the most versatile crops, and likely, is one of the oldest domesticated plants (Robinson, 1996; NFD, 2019). Different from marijuana (*Cannabis sativa forma indica*), hemp (*Cannabis sativa*) contains a low amount of delta-9 tetrahydrocannabinol ($\Delta 9$ -THC), a psychoactive constituent (>15 vs. $\leq 0.3\%$ in dry basis, respectively) but often a significant amount of cannabidiol (CBD), a non-psychoactive constituent with health benefits (Aluko, 2017).

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Hemp was historically a very important crop in the US, especially for the production of fiber (Robinson, 1996; Thompson et al., 1998); however, the close association with marijuana made this crop illegal in 1970 with the passage of the Comprehensive Drug Abuse Prevention and Control Act (Cherney and Small, 2016). The 2018 Farm Bill removed hemp from the Controlled Substances Act, classifying it as an agricultural product (US Congress, 2018). This has led to a flourishing hemp industry in US. Particularly important in the US is the cultivation of hemp for the extraction of CBD from the leaves of feminized hemp (i.e., without seed production) (Nichols, 2017), a process that generates a highly nutritive extracted byproduct that could be fed to livestock.

A review of the main features of hemp as a potential food alternative for dairy cows with discussion on the potential role of hemp as a means to help to improve the health and performance of cattle and the legal aspect of the research related to the use of hemp byproducts with livestock is presented.

Hemp for CBD Extraction is a Growing Industry in the US

Classification of hemp as an agricultural product by the 2018 Farm Bill prompted 38 states in the US to implement (or are in the process of implementing) a program for regulating industrial hemp allowing its cultivation. Hemp is a rapidly growing industry, with a value of \$3.7 billion globally in 2018 (NFD, 2019). The size of the industry is expected to reach \$26.6 billion by 2025 worldwide (Markets and Markets, 2019). Historically, the US has been a leading figure in the consumption of hemp products, with an exponential increase of import of hemp oil and seed in the last decade (Cherney and Small, 2016). In 2018, the US was the second biggest hemp market with \$1 billion in sales.

According to the USDA Crop Acreage Data Reported to FSA (USDA, 2020), in 2020 there were >68,000 acres cultivated on hemp in the USA, with 62% (42,400 acres) cultivated for CBD extraction, 16% for grain, 14% for seed, and 7% for fiber. The top 5 states for hemp cultivation were Montana (>10,000 acres), Colorado (8,700 acres), Kentucky, Minnesota, and Kansas (between 3,600 to 4,000 acres) (Figure 1). The large majority of hemp was cultivated for CBD extraction in all states except for Montana, Minnesota, and North Dakota. The amount of biomass produced in the US by the hemp used for CBD extraction can be somewhat estimated to be >25,000 US tons by considering that hemp cultivated for CBD can produce between 1,000 and 1,500 lb/acre of biomass. Thus, there is a very large amount of biomass that can be used, among others, in the diets of livestock and poultry. However, important questions remained to be answered before this can become a reality: What is the nutritive quality of such biomass? Any detrimental effects or benefits to the animals? Is it legal to feed this biomass to animals? What are the legal hurdles to overcome?

Use of Post-Extraction Hemp Biomass as Feed for Dairy Cows

Hemp can provide hemp seed, hempseed meal/cake, hemp seed oil, extracted hemp biomass, and whole hemp plant (including hemp flour) that can be used in animal diets. Hemp is rarely used as whole plant for animal feed (Kolodziejczyk et al., 2012). Chemical analysis of whole hemp plants from a study conducted in Europe indicated a relatively high dry matter (>90%), crude protein (21 to 27%) and ash content (14 to 20%) but low crude fiber (9 to 13%) (Suchy et al., 2011). A more recent analysis of the whole plant performed in the US (Kleinhenz et al., 2020a) reported somewhat different values, with a dry matter of 70%, 6.9%

crude protein, 81.6% NDF, and a relatively modest content of ash (8.8%). Chemical analysis of plant biomass collected from 3 types of hemp (fiber, seed, and dual-purpose) cultivated in the US were published in a MS thesis (Stringer, 2018). Throughout the growing season (from 30 to 156 day-after-planting), the nutritive value diminished with a decrease of crude protein (from 20 to 12%) and *in vitro* digestibility (from 85 to 60%) and an increase in NDF (from 35 to 55%) and ADF (from 25 to 40%). Overall, the data indicated that hemp might be useful as a forage for ruminants, especially in the earlier vegetative stages. However, the legal aspects related to the high content of cannabinoids associated with the value of extracting cannabinoids for the hemp industry, prompts to focus on post-cannabinoids extraction biomass (aka, spent hemp biomass or **SHB**) as feed for livestock rather than whole hemp plant.

Extraction of CBD

The extraction of CBD can be performed by several methods, including use of various solvents, such as hexane and ethanol, ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, cold pressing, supercritical fluid extraction, or a combination of them with a solvent (Fathordoobady et al., 2019). The most popular methods by industrial processors to extract cannabinoids in hemp are based on the use of cold ethanol or high-pressurized liquid CO₂. The latter can be used as such or in combination with a low amount of solvent (Fathordoobady et al., 2019). The low temperature during the extraction is important to control the decarboxylation of various cannabinoids (Fathordoobady et al., 2019). The use of solvent can be problematic if residuals of the solvent are left into the CBD oil or SHB. Thus, the use of CO₂ alone appears to be best method to obtain highly purified cannabinoids and SHB without residual of solvents.

Nutritive quality of SHB: proximate analysis

Because of the extraction protocol used to obtain high-quality CBD, the nutritive quality of SHB can be high. In addition, the plant is harvested at the flowering stage and the extraction is done mostly using flower and leaves, since those contain the highest concentration of cannabinoids, especially THC and CBD (Kleinhenz et al., 2020a).

An analysis of the nutritive values of the various parts of the hemp plant plus the extracted flower was provided recently by a group from Kansas State University (Kleinhenz et al., 2020a). According to the data presented in that study, hemp can be considered a decent feed for ruminants, but it appears to be somewhat deficient in energy. In the extracted flower biomass, the *in vitro* digestibility of the NDF was <20% at 30 hr; this is a poor digestibility, especially if compared to alfalfa which is >30% even for low digestible alfalfa (Fustini et al., 2017). According to the data presented by Kleinhenz and collaborators (Kleinhenz et al., 2020a), the hemp is very high in minerals, especially abundant is the level of calcium.

Nutritive values of SHB from 3 batches from 2 commercial processors in Oregon are available in Table 1. As a comparison, we reported also analysis of a commercial alfalfa meal and prior data on the post-extraction hemp flower (Kleinhenz et al., 2020a). Some of the parameters appears to be quite consistent between the 3 batches of SHB, including crude protein and the soluble crude proteins, which are relatively high and at par or even higher than alfalfa meal. High also are the values of crude fat and higher than alfalfa. However, the variation in crude fat is large and it is likely due to the different methods used for the extraction of cannabinoids by the 2 processors. One processor used cold ethanol (7.5% crude fat in the SHB)

and the other processor pressurized with CO₂ (between 2.9 to 4.3% crude fat).

Fiber content of SHB is similar to alfalfa meal (ca. 36%; Table 1); however, the values for the fiber are highly variable between the SHB from the 2 processors, with NDF ranging from 23.4 to 43.7%. The high level of NDF in 2 out of 3 SHB batches is somewhat surprising, considering that hemp for CBD extraction is harvested at the stage of full bloom, which should produce a biomass with low NDF. This was the case for the batch with 23.4% NDF, which was obtained from the processors that extracted only leaves and flowers and the hemp plants were likely in a more immature stage, while the 2 SHB with higher NDF values ($\geq 38\%$) were obtained from the other processor that harvested more mature plants and extracted CBD from the whole plant, including the stock (personal communication by the processors). The difference in fiber content between the SHB batches also affected the energy content of the biomass, with net energy for lactation ranging from 1.12 to 1.59 Mcal/kg. Considering the mean value of the evaluated SHB, the energy content is somewhat similar to alfalfa meal. Unfortunately, we did not measure the NDF digestibility in our samples to see if this is low as previously reported (Kleinhenz et al., 2020a).

As previously reported (Kleinhenz et al., 2020a), the SHB from our studies presented high amount of minerals (Table 1). Almost all minerals, both macro and micro, were more abundant in SHB compared to alfalfa meal. The high content of Ca in SHB could be of some concern to feed to dry dairy cows, especially Jersey cows, due to the importance of a low Ca diet during the prepartum to minimize the risk of milk fever (Oetzel, 2002). However, the level of K in the diet appears to be more important than the level of Ca in inducing metabolic alkalosis that can disrupt the Ca homeostasis and the

calculation of the dietary cation anion difference in the diet contains K in the numerator and not Ca (Goff and Horst, 1997). Potassium was one of the few minerals that was less abundant in SHB compared to alfalfa. The levels of microminerals are also of interest. Zn, Cu, and Mn were between 2- to 5-fold more abundant in SHB compared to alfalfa (Table 1). Those trace minerals are important for dairy cows, especially for their roles in the immune system and hoof health (Ballantine et al., 2002, Zhao et al., 2015, Faulkner et al., 2017).

Based on the above data, we could consider SHB a very good feed to be added to the diet of dry and lactating dairy cows. The nutritive data support the SHB to be an appropriate replacement for alfalfa. However, besides the classical nutritive values, a feed for dairy cows should be evaluated also for the content of amino acids and fatty acids, considering the importance of both for milk production and the quality of milk.

Nutritive quality of SHB: amino acid profiling

The amino acid composition of the SHB and alfalfa hay is reported in Table 2. The amino acid profiling of SHB and alfalfa is quite comparable, with few exceptions. Histidine and lysine are proportionally more abundant in alfalfa compared to SHB, while cysteine and glutamic acid are more abundant in SHB compared to alfalfa. Lysine is considered, together with methionine, among the most essential amino acids for milk synthesis (Robinson, 2010), although both the concentration of the 2 amino acids and their ratio are important to maximize milk protein synthesis (Awawdeh, 2016). A ratio of lysine/methionine of 3:1 has been demonstrated to maximize milk protein synthesis in dairy cows (Awawdeh, 2016; Wang et al., 2018). Interestingly, the SHB has a lysine/methionine ratio of exactly 3:1 (Table 2). Alfalfa

and SHB have similar abundance of essential and branched-chain amino acids. Those amino acids are important for their role in activating the mTOR pathway, the master regulator of milk protein synthesis but also for the synthesis of lactose and fat (Osorio et al., 2016). This is especially true for the branched-chain amino acid leucine, which its proportion in SHB is at par or slightly higher than alfalfa.

Nutritive quality of SHB: fatty acid profiling

The hemp seed and the derived oil are known to present high concentration of unsaturated fatty acids, especially $\omega 6$ (Rupasinghe et al., 2020). The content of fat in the SHB is relatively high (Table 1), despite the extensive extraction of cannabinoids and terpenes. The fatty acid profiling of SHB is presented in Figure 2. Similar to the oil, the hemp biomass is rich in unsaturated fatty acids ($60.4 \pm 0.26\%$); however, contrary to the oil, the $\omega 3$ is the highest in amount. This is not surprising since the SHB is made of plant materials and the thylakoid membranes where photosynthesis happens are rich in $\omega 3$ (Buccioni et al., 2012). The ratio $\omega 6/\omega 3$ in SHB is 0.60 ± 0.10 , which is highly desirable considering that this would affect the fatty acid profiling of the milk and a low $\omega 6/\omega 3$ is known to be important in preventing diseases in humans (Simopoulos, 2002), since $\omega 3$ fatty acid are used in cells as precursors for the synthesis of anti-inflammatory while $\omega 6$ pro-inflammatory eicosanoid lipid mediators (Calder, 2013). Therefore, the SHB appears to have a good fatty acid profile that is similar to what present in alfalfa (Figure 2).

Nutritive quality of SHB: antinutritional

We have assessed in the SHB also the potential presence of pesticides, mycotoxins, and heavy metals (data not showed). We did not detect any pesticide or mycotoxin above the limit

of quantitation and the amount of heavy metals was several fold below the maximum tolerable levels indicated by the Association of American Feed Control Officials and the National Research Council (Deemy, 2019).

Phytocannabinoids in SHB and Their Potential Effects on Health of Dairy Cows

Overall, the above data support the use of SHB as safe feed for dairy cows. The presence of cannabinoids can be also considered an important aspect to evaluate. Besides the legal aspects related to the content of cannabinoids, the presence of cannabinoids should be also considered in the light of their effects on animal health.

In the dairy industry, mastitis and diseases associated with the peripartum period are major concerns (LeBlanc et al., 2006). The peripartum is the most critical period for dairy cows due to large physiological and metabolic changes (Drackley, 1999). The cows during this period experience decreased feed intake, immune depression, and inflammatory-like conditions (Lopreiato et al., 2020, Pascottini et al., 2020). Excessive inflammatory conditions early post-partum can be detrimental to dairy cows, compromising their health and performance (Bionaz et al., 2007, Bradford et al., 2015). Thus, the use of alternative dietary approaches that improve the peripartum in high-producing dairy cows is a priority in the dairy industry. Furthermore, consideration of any alternative feedstuff for dairy cows, especially if it contains bioactive compounds, should be evaluated in the light of the health challenges associated with the peripartum period.

Phytocannabinoids are oxygen-containing C₂₁ aromatic hydrocarbons found in *Cannabis sativa L.* (Morales et al., 2017) with affinity for the cannabinoid receptors

(CB1 and CB2). The CBs are part of the endocannabinoid system, critical for many physiological processes, including appetite, pain-sensation, mood, and memory as observed in monogastrics (Ibsen et al., 2017, Wu, 2019). Besides CBs, more than 120 known phytocannabinoids can act on additional targets as previously reviewed (Morales et al., 2017). Among the phytocannabinoids, only Δ^9 -THC, and with lower affinity, Δ^8 -THC and CBN (cannabinol) can activate the CBs (Hanus et al., 2016). Phytocannabinoids present in *Cannabis sativa* have therapeutic potential in humans (Fraguas-Sanchez and Torres-Suarez, 2018), including treatment of chronic pain (Vuckovic et al., 2018), and have anti-inflammatory properties by binding the CB2 in immune cells (Nagarkatti et al., 2009); however, prolonged exposure to THC can have long-term detrimental consequences to health, especially to the central nervous system (Cohen et al., 2019).

Among phytocannabinoids, CBD does not seem to cause any detrimental effect on health, and it is known to have therapeutic properties to treat neural diseases and psychological disorders (Premoli et al., 2019). Recent evidence also suggests CBD acting as anti-inflammatory, anti-oxidative, and can positively affect the immune system (Olah et al., 2017; Jensen et al., 2018; Atalay et al., 2019; Vuolo et al., 2019). Although these data were produced in non-ruminant species, the modulation of the immune system, inflammation, and oxidative stress is of great interest for the dairy cows.

Cannabidiol is known to be a potent inhibition of the critical xenobiotics detoxification cytochrome P450 enzymes (Zendulka et al., 2016). Liver plays a central role in the early post-partum cows (Drackley, 1999, Cardoso et al., 2020) and a depression of P450 enzymes could be detrimental to this organ during the peripartum, considering the essential role in

detoxification of xenobiotics, including drugs. However, the reduction of P450 activity could potentially benefit fertility in dairy cows via decreased clearance of progesterone by the liver (Lemley et al., 2010) or reduced production of pro-inflammatory eicosanoids via P450 enzymes (Kuhn et al., 2020). Thus, it is important to determine the amount of CBD in SHB and its effect on dairy cows. CBD is the most abundant phytocannabinoid in hemp and its level varies greatly across varieties of hemp. Data available in the literature indicate amounts in order of 2 to 6 g/kg DM, while the Δ^9 -THC is between a trace and 0.3 g/kg DM (Hanus and Subova, 1989). Cannabinoids are quite low in the SHB we analyzed (Table 3), with the CBD being the most abundant (0.3 to 0.7%) for a total cannabinoids level of 1.6% (0.6 to 3%). The THC is present at very low amount in SHB. Of the 3 SHB analyzed, only one had a detectable level of THC with a total THC of 0.07% (with 0.03% Δ^9 -THC). Thus, the SHB presents an overall favorable composition of cannabinoids for the use as feed for dairy cows (i.e., overall low cannabinoids but also high CBD and low THC).

Besides cannabinoids, hemp also contains a large amount of polyphenols with antioxidant properties and other secondary metabolites (Flores-Sanchez and Verpoorte, 2008; Teh and Birch, 2014). The above strongly indicate the possibility of using hemp as a nutraceutical in dairy cows, besides being used as a forage.

An experiment was carried out recently at Oregon State University with the aim to assess the effect of SHB on animal health and performance (unpublished data). In the experiment, Polypay finishing rams were fed with 10 or 20% SHB as alternative to alfalfa meal for 2 months, with half of the animals in each group having the SHB withdrawn for

1 month. Preliminary data generated from the experiment reveal that SHB up to 20% is safe to be fed to ruminants with no detrimental effects on health or performance. The rams fed with SHB had a significant increase in feed intake compared to the control group, especially when fed for >1 month and with a dose of 10% SHB; however, the rams fed with 20% SHB had a depressed feed intake, especially during the first month of the experiment. We observed a refusal to eat pelletized SHB by >30% of the animals. However, the grinding of the pelletized SHB and subsequent mixing with the grain was sufficient to have 100% of the animals eating SHB. We initially thought that the refusal of eating by the animals was due to the content of terpenes; however, upon analysis, no terpenes were detected above the limit of quantitation. We also observed a linear effect of SHB feeding to increase bilirubin in the blood, indicating a decreased clearance by the liver, likely due to the known inhibition of the P450 enzymes by CBD (Zendulka et al., 2016). Despite the inhibition of the bilirubin clearance, the data did not indicate any damage to the liver. Furthermore, we observed an increase in testicles weight and number and activity of sperms in animals fed SHB. Overall, our initial data support the safe use of SHB to feed ruminants and even suggest some potential benefits.

In recent work performed at Kansas State University, a single dose of SHB was provided to castrated male Holstein calves to assess the pharmacokinetics of cannabinoids (Kleinhenz et al., 2020b). The researchers did not observe any issue on health or behavior of the animals.

The data generated from the experiments performed in monogastric animals, the data generated in the experiment performed at Oregon State University in rams - especially the stimulation of feed intake, the experiment performed at Kansas State University in

dairy calves, and the anti-inflammatory and anti-oxidative potential of CBD, allow the conclusion that the use of SHB is not only safe, but it might be a promising nutraceutical in dairy cows. Nutraceutical approaches to improve the transition from pregnancy to lactation in dairy cows have been previously reviewed (Lopreiato et al., 2020). The use of hemp as nutraceutical has also been proposed for humans (Rupasinghe et al., 2020).

Legalization of SHB as Feed for Dairy Cows

Despite the overall positive results of the few animal feeding trials briefly reviewed above about the use of hempseed cake and the good nutritive quality and apparent safety of SHB in our experiments, the use of those hemp byproducts are yet to be legalized in the USA. The main issue associated with the use of those byproducts is the content of cannabinoids, especially THC and CBD, as clearly stated by the FDA (FDA, 2018):

“[...] because both CBD and THC are active ingredients in FDA-approved drugs and were the subject of substantial clinical investigations before they were marketed as foods or dietary supplements. Under the FD&C Act, it’s illegal to introduce drug ingredients like these into the food supply, or to market them as dietary supplements.”

In a web-page instituted for Q&A about use of cannabis-derived products, the FDA states [response to question 10 (FDA, 2019)]:

“[...] in the case of animal feed, [...] the drug is a new animal drug approved for use in feed and used according to the approved labeling. However, based on available evidence, FDA has concluded that none of these is the case for THC or CBD.”

Thus, there is an urgent need to produce data to inform the approval process by the FDA-CVM. The agencies would greatly benefit from data about the residuals of cannabinoids, particularly THC and CBD, in the products of animals fed with SHB or other hemp byproducts. One additional concern, especially for the CVM, is the effect of those byproducts on the health of the animals. One major issue related to the decision of FDA-CVM to legalize the use of those byproducts is the absence in the US of a tolerable dose intake (**TDI**) for CBD and THC. The TDI is the maximum dose of a compound that can be consumed daily over a lifetime without appreciable health risk. There are not yet data on the residuals of cannabinoids in meat and milk of animals fed to livestock.

THC transfer in milk and meat

Although there are not yet data available on the presence of cannabinoids residuals in milk, it is possible to provide some estimates. Contrary to studies in humans and other animals consuming marijuana (LactMed, 2006; Garry et al., 2009), studies of THC transfer into milk in ruminants are scant. There is a study from 1974 in lactating sheep where it was determined that THC is secreted in milk at a rate of 0.04% (Jakubovič et al., 1974). To our knowledge, there are only 2 studies on the transfer/amount of THC in milk when hemp or its byproducts are fed to dairy cows, as summarized by the European Food Safety Authority report (EFSA, 2011). Based on those data, the EFSA adopted a transfer rate of ingested THC to milk of 0.15%. Based on that transfer, it was estimated that feeding as low as 0.5 kg/day of hemp (as whole plant) containing 0.2% THC to a cow producing 35 kg/day of milk, the exposure to THC by people drinking the milk was above the TDI, considered to be 0.4 µg/kg of BW. However, the EFSA estimated that the potential accumulation of THC in milk of cows fed hempseed or hempseed

cake containing 0.0012% THC is significantly below the TDI; thus, they concluded that the use of hemp seed or hempseed cake with dairy cows is safe for consumers.

Based on the above data, we can estimate the possible transfer of THC to milk by feeding SHB. Assuming a diet for mid-lactation Jersey dairy cows with 20% SHB with 300 mg THC/kg DM, a transfer to milk of 0.15%, 20 kg of DM fed daily, and 30 kg of milk produced daily, the amount of THC residual in milk would be 45 ng/mL, which is above the 1.5 ng/mL limit of detection of the most recent methods used to detect THC in milk (Escrivá et al., 2017). Thus, THC should be detectable in milk of cows fed SHB. If that milk were fed to a child of 15 kg of body weight and drinking 3 cups of milk a day, the child would be exposed to 2.3 µg THC/kg BW. This is above the TDI of 0.4 µg THC/kg BW indicated by the European report (EFSA, 2011). For an adult weighing 60 kg and consuming 3 cups of milk daily, it would be 0.56 µg THC/kg, i.e., still above that TDI. However, the Australian and New Zealand governments have indicated a TDI for THC of 6 µg/kg BW (FSANZ, 2014). With that TDI, the THC present in milk would be considered safe for both adults and children.

As indicated above, there is not a TDI set for THC in the United States. Prior data generated in monogastrics using THC can be of help in this direction. Mice consuming a dough with 1 mg/kg of THC (i.e., 5 µg THC/kg of BW) had significant decreased locomotor activity (Smoker et al., 2019). Clinical trials using daily doses of THC up to 15 mg (i.e., 26 µg/kg BW) detected little side effects (Hillen et al., 2019) and doses of 2 mg/day of THC (i.e., 3.4 µg/kg BW) of a cannabis decoction had no detrimental effects in humans (Pellesi et al., 2018). According to those data, the potential dose of THC in milk of cows with SHB should be safe to be fed to humans.

There are not studies on the accumulation of THC in tissues by feeding hemp. Injection of 200 µg of THC/kg of BW in pigs demonstrated an acute accumulation in tissues, but a quick disappearance in the following 24 hr, except for fat tissue, and to lower degree, lung where it persisted (Brunet et al., 2006). Work performed in lactating sheep indicated a transfer of THC to tissue of approximately 85% (Jakubovič et al., 1974) and the bioavailability (using monogastric data) is 12% (McGilveray, 2005). Using those data with cattle, feeding 20% SHB with 0.03% CBD to Jersey dairy cows weighing 500 kg and eating 20 kg/day of DM, the amount of THC in meat would be 245 µg/kg. An adult human of 60 kg BW eating 2 burgers of 6 oz. each (ca. 240 g of meat) would be exposed to 0.98 µg/kg BW, above the TDI from EFSA but below the one from Australia and New Zealand.

CBD transfer in milk and meat

There are no studies on the transfer of CBD to milk or meat. However, using the same numbers as for THC, we can estimate that milk from dairy cows fed 20% of SHB containing 1% CBD would have 1.5 µg/mL of CBD. Three cups of milk will provide approximately 1.12 mg CBD or 18.8 µg CBD/kg of BW. Using the same criteria and calculations as for the THC, we expect to have 8.2 mg of CBD/kg of meat, which would result in 32.6 µg/kg BW for an adult eating 2 burgers.

In animal studies, 10 mg/kg BW of CBD injected can produce toxicity, but doses had to be higher to produce toxicity when provided orally with >50 mg/kg BW to observe any adverse effect (Huestis et al., 2019). The dose of CBD potentially present in milk or meat would be between 500- and >1000-fold below the 20 mg/kg/day recommended dose for the FDA-approved Epidiolex[®], the 98% pharmaceutical grade CBD available commercially and used for

the treatment of epilepsy in pediatric patients (Huestis et al., 2019) and >2000-fold lower than the dose of CBD that can produce toxicity. Thus, the amount of CBD potentially present in milk or meat should be of little concern for human health.

A recent study was performed to assess the pharmacokinetics of cannabinoids present in SHB using dairy calves (Kleinhenz et al., 2020b). The animals were fed with a single oral exposure of SHB to reach a dose of 5.4 mg/kg BW of CBDA. The authors observed a readily absorption of cannabinoids by the animals, with detection of cannabinoids in blood few minutes after dosage and a peak concentration in blood between 12 and 24 hr post-dosage. The data from the study also revealed some differences in absorption of cannabinoids. Despite almost 30-fold lower cannabidiolic acid (CBDA) in SHB, the level in blood was similar to THCA-A and CBCA, especially during the first 24 hr post-dosage. Those data indicate the need to study in vivo the transfer of various cannabinoids in milk and meat in animals fed SHB.

Legal aspects in conducting research with SHB

The legal status of SHB makes research on live animals difficult. According to FDA, the animals exposed to SHB cannot enter the food chain (FDA, 2018, 2019). Thus, it is a requirement that animals used in those experiments be euthanized and landfilled at the end of the experiments. This can provide the opportunity to collect more samples for analyses; however, at the same time, the above requirement increases the cost of research. There is, however, the possibility of requesting a Food Use Authorization to the FDA to avoid the euthanasia at the end of the experiments (Office of New Animal Drug Evaluation, 2021).

Conclusions

The review of the available scientific literature and the data on nutritive values and abundance of cannabinoids, as well the estimated potential transfer of cannabinoids in milk and meat, support the safe use of SHB as feed alternative/supplement for dairy cows. We are still a long way to be able to feed SHB to dairy cows. We still need to provide data for the FDA-CVM to initiate the legalization of SHB as feed for livestock and poultry. For this, it is imperative to assess the presence of residuals of cannabinoids in milk and meat by studies performed *in vivo*. We still need to evaluate fully the effect of SHB on animal health and performance of dairy cows, although the initial data indicate a positive effect of SHB on ruminants. With the intent to close the above gap in knowledge, a project was just awarded by the USDA NIFA CARE (project number ORE01002) to study the effect of feeding SHB on dairy cows and the cannabinoids residuals in milk, muscle, and adipose tissue. Finally, it is of extreme interest for the dairy industry on the potential use of SHB as a nutraceutical; however, as for the above, this potential needs to be fully elucidated via sound scientific research.

References

Aluko, R.E. 2017. Chapter 7 - Hemp Seed (*Cannabis sativa* L.) Proteins: Composition, Structure, Enzymatic Modification, and Functional or Bioactive Properties. Pages 121-132 in *Sustainable Protein Sources*. S. R. Nadathur, J.P.D. Wanasundara, and L. Scanlin, ed. Academic Press, San Diego.

Atalay, S., I. Jarocka-Karpowicz, and E. Skrzydlewska. 2019. Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants* (Basel, Switzerland) 9(1):21.

Awawdeh, M.S. 2016. Rumen-protected methionine and lysine: Effects on milk production and plasma amino acids of dairy cows with reference to metabolisable protein status. *Journal of Dairy Research* 83(2):151-155.

Ballantine, H.T., M.T. Socha, D.A. D.J. Tomlinson, A.B. Johnson, A.S. Fielding, J.K. Shearer, and S.R. Van Amstel. 2002. Effects of feeding complexed zinc, manganese, copper, and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction, and lactation performance. *Professional Animal Scientist* 18(3):211-218.

Bharathan, M., D.J. Schingoethe, A.R. Hippen, K.F. Kalscheur, M.L. Gibson, and K. Karges. 2008. Conjugated linoleic acid increases in milk from cows fed condensed corn distillers solubles and fish oil. *J. Dairy Sci.* 91(7):2796-2807.

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. *J. Dairy Sci.* 90(4):1740-1750.

Bradford, B.J., K. Yuan, J.K. Farney, L.K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J. Dairy Sci.* 98(10):6631-6650.

Brunet, B., C. Doucet, N. Venisse, T. Hauet, W. Hébrard, Y. Papet, G. Mauco, and P. Mura. 2006. Validation of Large White Pig as an animal model for the study of cannabinoids metabolism: Application to the study of THC distribution in tissues. *Forensic Science International* 161(2):169-174.

- Buccioni, A., M. Decandia, S. Minieri, G. Molle, and A. Cabiddu. 2012. Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. *Animal Feed Science and Technology* 174(1):1-25.
- Calder, P.C. 2013. Omega-3 polyunsaturated fatty acids and inflammatory processes: Nutrition or pharmacology? *British Journal Clinical Pharmacology* 75(3):645-662.
- Cardoso, F.C., K.F. Kalscheur, and J.K. Drackley. 2020. Symposium review: Nutrition strategies for improved health, production, and fertility during the transition period. *J. Dairy Sci.* 103(6):5684-5693.
- Cherney, J.H. and E. Small. 2016. Industrial hemp in North America: Production, politics and potential. *Agronomy-Basel* 6(4).
- Cohen, K., A. Weizman, and A. Weinstein. 2019. Positive and negative effects of cannabis and cannabinoids on health. *Clin. Pharmacol. Ther.* 105(5):1139-1147.
- Deemy, M. 2019. Report on heavy metals in animal food. Vol. 2021. FDA-CVM.
- Drackley, J.K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82(11):2259-2273.
- EFSA. 2011. Scientific opinion on the safety of hemp (*Cannabis* genus) for use as animal feed. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). *Efsa J* 9(3).
- Escrivá, Ú., M.J. Andrés-Costa, V. Andreu, and Y. Picó. 2017. Analysis of cannabinoids by liquid chromatography–mass spectrometry in milk, liver and hemp seed to ensure food safety. *Food Chemistry* 228:177-185.
- Fathordoobady, F., A. Singh, D.D. Kitts, and A.P. Singh. 2019. Hemp (*Cannabis Sativa* L.) extract: Anti-microbial properties, methods of extraction, and potential oral delivery. *Food Reviews International* 35(7):664-684.
- Faulkner, M.J., B.A. Wenner, L.M. Solden, and W.P. Weiss. 2017. Source of supplemental dietary copper, zinc, and manganese affects fecal microbial relative abundance in lactating dairy cows. *J. Dairy Sci.* 100(2):1037-1044.
- FDA. 2018. Statement from FDA Commissioner Scott Gottlieb, M.D., on signing of the Agriculture Improvement Act and the agency's regulation of products containing cannabis and cannabis-derived compounds. Vol. 2019. S. Gottlieb, ed. Commissioner of Food and Drugs - Food and Drug Administration.
- FDA. 2019. FDA Regulation of Cannabis and Cannabis-Derived Products: Questions and Answers. Vol. 2019. S. Gottlieb, ed. Commissioner of Food and Drugs - Food and Drug Administration.
- Flores-Sanchez, I.J. and R. Verpoorte. 2008. Secondary metabolism in cannabis. *Phytochemistry Reviews* 7(3):615-639.
- Fraguas-Sanchez, A.I. and A.I. Torres-Suarez. 2018. Medical use of cannabinoids. *Drugs* 78(16):1665-1703.
- FSANZ. 2014. Application A1039 - Low THC Hemp as a Food. in A1039. F. S. A. N. Zealand, ed.

- Fustini, M., A. Palmonari, G. Canestrari, E. Bonfante, L. Mammi, M.T. Pacchioli, G.C. J. Sniffen, R.J. Grant, K.W. Cotanch, and A. Formigoni. 2017. Effect of undigested neutral detergent fiber content of alfalfa hay on lactating dairy cows: Feeding behavior, fiber digestibility, and lactation performance. *J. Dairy Sci.* 100(6):4475-4483.
- Garry, A., V. Rigourd, A. Amirouche, V. Fauroux, S. Aubry, and R. Serreau. 2009. Cannabis and breastfeeding. *J. Toxicol.* 2009:596149.
- Goff, J.P. and R.L. Horst. 1997. Effects of the addition of potassium or sodium, but not calcium, to prepartum ratios on milk fever in dairy cows. *J. Dairy Sci.* 80(1):176-186.
- Hanus, L.O., S.M. Meyer, E. Munoz, O. Taglialatela-Scafati, and G. Appendino. 2016. Phytocannabinoids: A unified critical inventory. *Nat. Prod. Rep.* 33(12):1357-1392.
- Hanus, L. and D. Subova. 1989. The amount of main cannabinoid substances in hemp, cultivated for industrial fibre production and their changes in the course of one vegetation period. *Acta Univ. Palacki. Olomuc. Fac. Med.* 122:11-23.
- Hillen, J.B., N. Soulsby, C. Alderman, and G.E. Caughey. 2019. Safety and effectiveness of cannabinoids for the treatment of neuropsychiatric symptoms in dementia: A systematic review. *Ther. Adv. Drug Saf.* 10:2042098619846993.
- Huestis, M.A., R. Solimini, S. Pichini, R. Pacifici, J. Carlier, and F.P. Busardò. 2019. Cannabidiol adverse effects and toxicity. *Curr. Neuropharmacol.* 17(10):974-989.
- Ibsen, M.S., M. Connor, and M. Glass. 2017. Cannabinoid CB1 and CB2 receptor signaling and bias. *Cannabis Cannabinoid Res.* 2(1):48-60.
- Jakubovič, A., R.M. Tait, and P.L. McGeer. 1974. Excretion of THC and its metabolites in ewes' milk. *Toxicology and Applied Pharmacology* 28(1):38-43.
- Jensen, H.M., R. Korbut, P.W. Kania, and K. Buchmann. 2018. Cannabidiol effects on behaviour and immune gene expression in zebrafish (*Danio rerio*). *PLoS One* 13(7):e0200016.
- Kleinhenz, M.D., G. Magnin, S.M. Ensley, J.J. Griffin, J. Goeser, E. Lynch, and J.F. Coetzee. 2020a. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. *Applied Animal Science* 36(4):489-494.
- Kleinhenz, M.D., G. Magnin, Z. Lin, J. Griffin, K.E. Kleinhenz, S. Montgomery, A. Curtis, M. Martin, and J. F. Coetzee. 2020b. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (*Cannabis sativa*). *Sci. Rep.* 10(1):12753.
- Kolodziejczyk, P., L. Ozimek, and J. Kozłowska. 2012. The application of flax and hemp seeds in food, animal feed and cosmetics production. Pages 329–366 in *Handbook of Natural Fibres*. Woodhead Publishing Limited.
- Kuhn, M.J., A.K. Putman, and L.M. Sordillo. 2020. Widespread basal cytochrome P450 expression in extrahepatic bovine tissues and isolated cells. *J. Dairy Sci.* 103(1):625-637.
- LactMed. 2006. Cannabis. In *Drugs and Lactation Database (LactMed)*. Bethesda (MD). LeBlanc, S. J., K. D. Lissemore, D. F. Kelton, T. F. Duffield, and K. E. Leslie. 2006. Major advances in disease prevention in dairy cattle. *J. Dairy Sci.* 89(4):1267-1279.

- Lemley, C.O., T.A. Wilmoth, L.R. Tager, K.M. Krause, and M.E. Wilson. 2010. Effect of a high cornstarch diet on hepatic cytochrome P450 2C and 3A activity and progesterone half-life in dairy cows. *J. Dairy Sci.* 93(3):1012-1021.
- Lopreiato, V., M. Mezzetti, L. Cattaneo, G. Ferronato, A. Minuti, and E. Trevisi. 2020. Role of nutraceuticals during the transition period of dairy cows: A review. *J. Anim. Sci. and Biotechnology* 11(1):96.
- Markets and Markets. 2019. Industrial Hemp Market. Vol. 2021. Markets and Markets™.
- McGilveray, I.J. 2005. Pharmacokinetics of cannabinoids. *Pain Res. Manag.* 10 Suppl A:15A-22A.
- Morales, P., D.P. Hurst, and P.H. Reggio. 2017. Molecular targets of the phytocannabinoids: A complex picture. *Prog. Chem. Org. Nat. Prod.* 103:103-131.
- Nagarkatti, P., R. Pandey, S.A. Rieder, V.L. Hegde, and M. Nagarkatti. 2009. Cannabinoids as novel anti-inflammatory drugs. *Future Medicinal Chemistry* 1(7):1333-1349.
- NFD. 2019. The Global State of Hemp: 2019 Industry Outlook. in *Hemp Business Journal*.
- Nichols, K. 2017. Hemp Report: TOP 10 U.S. STATES. H. I. Daily, ed.
- Oetzel, G.R. 2002. Diseases of dairy animals, Noninfectious | Milk Fever. Pages 824-830 in *Encyclopedia of Dairy Sciences*. H. Roginski, ed. Elsevier, Oxford.
- Office of New Animal Drug Evaluation. 2021. Investigational food-use authorizations: The role of the target animal division reviewer. P.P.A.P. Manual, ed. Center for Veterinary Medicine FDA-CVM.
- Olah, A., Z. Szekanecz, and T. Biro. 2017. Targeting cannabinoid signaling in the immune system: "High"-ly exciting questions, possibilities, and challenges. *Front. Immunol.* 8:1487.
- Osorio, J.S., J. Lohakare, and M. Bionaz. 2016. Biosynthesis of milk fat, protein, and lactose: Roles of transcriptional and posttranscriptional regulation. *Physiol. Genomics* 48(4):231-256.
- Pascottini, O.B., J.L.M.R. Leroy, and G. Opsomer. 2020. Metabolic stress in the transition period of dairy cows: Focusing on the prepartum period. *Animals : an open access journal from MDPI* 10(8):1419.
- Pellesi, L., M. Licata, P. Verri, D. Vandelli, F. Palazzoli, F. Marchesi, M.M. Cainazzo, L.A. Pini, and S. Guerzoni. 2018. Pharmacokinetics and tolerability of oral cannabis preparations in patients with medication overuse headache (MOH) - A pilot study. *Eur. J. Clin. Pharmacol.* 74(11):1427-1436.
- Premoli, M., F. Aria, S.A. Bonini, G. Maccarinelli, A. Gianoncelli, S.D. Pina, S. Tambaro, M. Memo, and A. Mastinu. 2019. Cannabidiol: Recent advances and new insights for neuropsychiatric disorders treatment. *Life Sci.* 224:120-127.
- Ribeiro, C.V.D.M., S.K.R. Karnati, and M.L. Eastridge. 2005. Biohydrogenation of fatty acids and digestibility of fresh alfalfa or alfalfa hay plus sucrose in continuous culture. *J. Dairy Sci.* 88(11):4007-4017.
- Robinson, R. 1996. *The Great Book of Hemp: The Complete Guide to the Environmental, Commercial, and Medicinal Uses of the World's Most Extraordinary Plant*. Park Street Press, Rochester, Vermont.

- Robinson, P.H. 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: A systematic review of the literature. *Livestock Science* 127(2):115-126.
- Rupasinghe, H.P.V., A. Davis, S.K. Kumar, B. Murray, and V.D. Zheljzkov. 2020. Industrial hemp (*Cannabis sativa subsp. sativa*) as an emerging source for value-added functional food ingredients and nutraceuticals. *Molecules* 25(18).
- Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* 56(8):365-379.
- Smoker, M.P., K. Mackie, C.C. Lapiush, and S.L. Boehm, 2nd. 2019. Self-administration of edible delta(9)-tetrahydrocannabinol and associated behavioral effects in mice. *Drug Alcohol Depend* 199:106-115.
- Stringer, C. E. 2018. Evaluating hemp (*Cannabis Sativa*) as a forage based on yield, nutritive analysis, and morphological composition. *Plant and Soil Sciences*. Vol. Master of Science (MS). University of Kentucky.
- Suchy, P., E. Strakova, V. Vecerek, N. Mas, V. Serman, and I. Herzig. 2011. Hemp (*Cannabis SAtiva*) and the possibility of its use as animal feed. *Krmiva* 53(1):17-24.
- Teh, S.-S. and E.J. Birch. 2014. Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes. *Ultrasonics Sonochemistry* 21(1):346-353.
- Thompson, E.C., M.C. Berger, and S.N. Allen. 1998. Economic Impact of Industrial Hemp in Kentucky. C. f. B. a. E. R. U. o. Kentucky, ed. University of Kentucky.
- Toral, P., G. Hervás, H. Missaoui, S. Andrés, F. Giráldez, S. Jellali, and P. Frutos. 2016. Effects of a tannin-rich legume (*Onobrychis viciifolia*) on in vitro ruminal biohydrogenation and fermentation. *Spanish Journal of Agricultural Research* 14:e0602.
- US Congress. 2018. S.2667 - Hemp Farming Act of 2018.
- USDA. 2020. FSA Crop Acreage Data Reported to FSA: 2020.
- Vuckovic, S., D. Srebro, K.S. Vujovic, C. Vucetic, and M. Prostran. 2018. Cannabinoids and Pain: New Insights From Old Molecules. *Front. Pharmacol.* 9:1259.
- Vuolo, F., S.C. Abreu, M. Michels, D.G. Xisto, N.G. Blanco, J.E. Hallak, A.W. Zuardi, J.A. Crippa, C. Reis, M. Bahl, E. Pizzichinni, R. Maurici, M.M.M. Pizzichinni, P.R.M. Rocco, and F. Dal-Pizzol. 2019. Cannabidiol reduces airway inflammation and fibrosis in experimental allergic asthma. *Eur. J. Pharmacol.* 843:251-259.
- Wang, B., L.S. Jiang, and J.X. Liu. 2018. Amino acid profiles of rumen undegradable protein: A comparison between forages including cereal straws and alfalfa and their respective total mixed rations. *J. Anim. Physiol. Anim. Nutr. (Berl)* 102(3):601-610.
- Wu, J. 2019. Cannabis, cannabinoid receptors, and endocannabinoid system: Yesterday, today, and tomorrow. *Acta Pharmacol. Sin.* 40(3):297-299.
- Zendulka, O., G. Dovrtelova, K. Noskova, M. Turjap, A. Sulcova, L. Hanus, and J. Jurica. 2016. Cannabinoids and cytochrome P450 interactions. *Curr. Drug Metab.* 17(3):206-226.

Zhao, X.-J., Z.-P. Li, J.-H. Wang, X.-M. Xing, Z.-Y. Wang, L. Wang, and Z.-H. Wang. 2015. Effects of chelated Zn/Cu/Mn on redox status, immune responses and hoof health in lactating Holstein cows. *Journal of Veterinary Science* 16(4):439-446.



Table 1. Nutritive values of post-CBD extracted hemp (SHB) from 3 batches obtained from 2 processors in Oregon. Reported are the mean and standard deviation (SD). The values for SHB are compared to a commercial alfalfa meal and prior published values for post-CBD extraction hemp flower biomass (Kleinhenz et al., 2020a). The analysis of SHB and alfalfa meal was performed by a commercial laboratory (Cumberland Valley Analytical Services, PA)

| Component | SHB | | Extracted Flower | Alfalfa Meal |
|-------------------------------------|------|------|------------------|--------------|
| | Mean | SD | | |
| Dry Matter, % | 92.5 | 2.7 | 96.6 | 90.9 |
| Crude protein, % DM | 21.0 | 1.7 | 24.5 | 20.8 |
| Soluble Protein, % CP | 36.2 | 7.5 | 21.4 | 31.0 |
| Acid Detergent Fiber, % DM | 29.0 | 10.1 | 18.1 | 30.8 |
| Neutral Detergent Fiber, % DM | 35.7 | 10.8 | 30.9 | 36.9 |
| Non-Fiber Carbohydrate, % DM | 28.3 | 8.2 | 20.2 | 30.3 |
| Crude fat, % DM | 4.9 | 2.4 | 3.2 | 1.6 |
| Ash, % DM | 16.1 | 0.27 | 25.7 | 10.0 |
| % Ca, % DM | 3.07 | 0.03 | 3.6 | 1.87 |
| %P, % DM | 0.73 | 0.06 | 0.4 | 0.34 |
| %Mg, % DM | 0.57 | 0.07 | 0.5 | 0.42 |
| %K, % DM | 2.24 | 0.02 | 1.9 | 3.15 |
| %Na, % DM | 0.04 | 0.27 | | 0.13 |
| Fe, ppm | 729 | 343 | | 951 |
| Zn, ppm | 81.0 | 14.9 | | 18.0 |
| Cu, ppm | 19.0 | 4.6 | | 12.0 |
| Mn, ppm | 202 | 93 | | 71 |
| Total Digestible Nutrients, % | 58 | 10 | 46 | 61 |
| Metabolizable, Mcal/kg | 2.14 | 0.5 | | 2.39 |
| Net Energy for Lactation, Mcal/kg | 1.30 | 0.3 | | 1.36 |
| Net Energy for Maintenance, Mcal/kg | 1.27 | 0.4 | | 1.43 |
| Net Energy for Growth, Mcal/kg | 0.70 | 0.4 | | 0.85 |

Table 2. Amino acid composition (as % of all amino acids) of post-CBD extracted hemp (SHB) from 3 batches obtained from 2 processors in Oregon. Reported are the mean and standard deviation (SD). The values for SHB are compared to previously published values of alfalfa hay (Wang et al., 2018). The analysis of SHB was performed by a commercial laboratory (Cumberland Valley Analytical Services, PA).

| Amino Acid | SHB | | Alfalfa |
|-------------------|------|------|---------|
| | Mean | SD | |
| Alanine | 5.9 | 0.50 | 4.5 |
| Arginine | 6.2 | 0.37 | 4.4 |
| Aspartic acid | 15.5 | 1.19 | 20.9 |
| Cysteine | 1.7 | 0.05 | 1.0 |
| Glutamic acid | 12.4 | 0.16 | 7.6 |
| Glycine | 6.2 | 0.46 | 4.9 |
| Histidine | 2.0 | 0.31 | 3.0 |
| Isoleucine | 4.6 | 0.43 | 4.9 |
| Leucine | 7.6 | 0.36 | 7.0 |
| Lysine | 4.7 | 1.06 | 7.1 |
| Methionine | 1.5 | 0.22 | 1.4 |
| Phenylalanine | 5.4 | 0.24 | 5.9 |
| Proline | 5.8 | 0.37 | 7.5 |
| Serine | 5.2 | 0.54 | 5.9 |
| Threonine | 4.3 | 0.18 | 4.8 |
| Tyrosine | 3.4 | 0.16 | 2.9 |
| Valine | 5.8 | 0.37 | 6.3 |
| Lys/Met | 3.0 | 0.24 | 5.1 |
| EAA ¹ | 43.9 | 0.57 | 45.0 |
| BCAA ¹ | 18.0 | 1.10 | 18.0 |

¹Essential amino acids (Arg, His, Leu, Ile, Lys, Met, Phe, Thr, Tyr, and Val) and branched-chain amino acids (Leu, Ile, Val).

Table 3. Cannabinoids concentration of post-CBD extracted hemp (SHB) from 3 batches obtained from 2 processors in Oregon. Reported are the mean and standard deviation (SD) of % cannabinoid (in wet basis). The analysis was performed by a commercial laboratory (Columbia Laboratories, Tentamus, OR).

| Analyte | Mean | SD |
|--------------------|------|-------|
| CBC | 0.04 | 0.035 |
| CBC-A | 0.10 | 0.144 |
| CBC-Total | 0.13 | 0.158 |
| CBD | 0.52 | 0.189 |
| CBD-A | 0.86 | 0.885 |
| CBD-Total | 1.28 | 0.869 |
| CBDV | <LoQ | |
| CBDV-A | <LoQ | |
| CBDV-Total | <LoQ | |
| CBG | <LoQ | |
| CBG-A | 0.03 | 0.060 |
| CBG-Total | 0.03 | 0.052 |
| CBL | <LoQ | |
| CBN | <LoQ | |
| D8-THC | <LoQ | |
| D9-THC | 0.01 | 0.018 |
| THC-A | 0.01 | 0.023 |
| THC-Total | 0.02 | 0.038 |
| THCV | <LoQ | |
| THCV-A | <LoQ | |
| THCV-Total | <LoQ | |
| Total Cannabinoids | 1.59 | 1.267 |

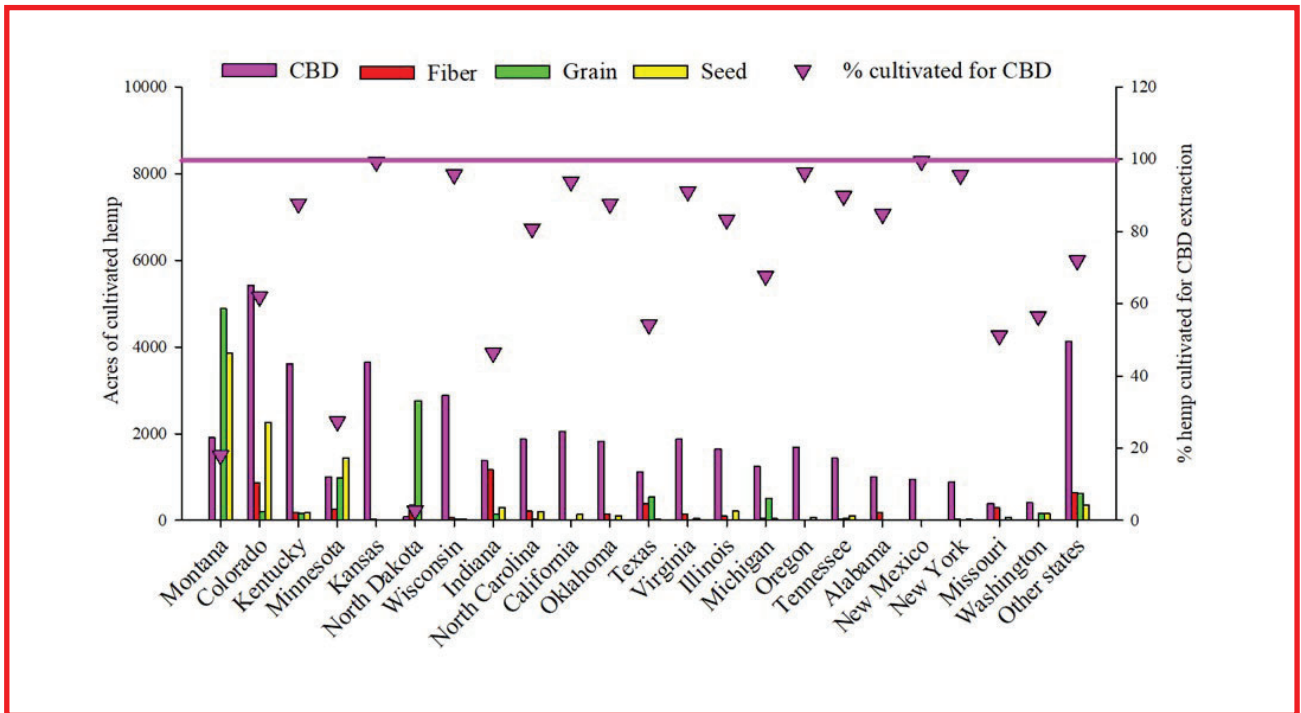


Figure 1. Hemp cultivation in USA in 2020 according to the USDA Crop Acreage Data Reported to FSA (USDA, 2020). Reported are the acres for cultivation of hemp based on their utilization: cannabidiol extraction (CBD), fiber, grain, and seed. Reported is also the % proportion of hemp cultivated for CBD extraction. The horizontal purple line denotes the total proportion of hemp cultivation (i.e., 100%). States are sorted from left to right based on the total amount of acres of hemp cultivated.



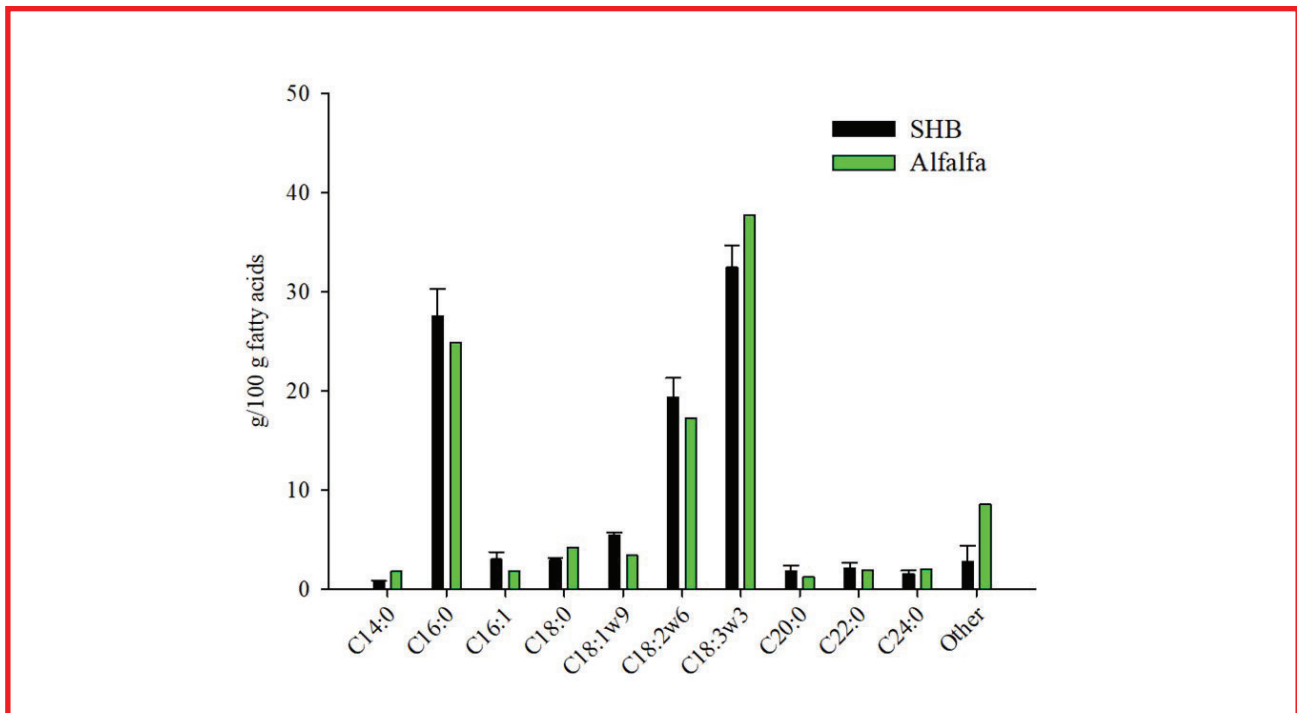


Figure 2. Fatty acid profiling of post-CBD extracted hemp (SHB) from 3 batches obtained from 2 processors in Oregon. Reported are the mean and standard deviation (SD). The fatty acid profiling analysis of the SHB was performed by a commercial laboratory (Cumberland Valley Analytical Services, PA). As comparison, mean of previously published data on fatty acid profiling of alfalfa is reported (Ribeiro et al., 2005; Bharathan et al., 2008; Toral et al., 2016).