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Effect of Dietary Hempseed Meal on Growth Performance, Feed Efficiency and Blood Parameters in Yearling Rough Stock Bulls

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Simple Summary: Industrial hemp by-products have yet to be approved by the organizations governing what can be considered an animal feed ingredient, highlighting the importance of determining the safety and efficacy of hempseed meal as a potential feed ingredient for ruminants. In this study, hempseed meal, a by-product of hemp oil production, was offered to yearling rough-stock bulls at 10% of the diet. No effects on weight, interim intake, or efficiency were observed, suggesting that the overall performance of animals offered the hempseed meal versus a control supplement was not different. Bulls offered the hempseed meal supplement were also observed to maintain an elevated dry matter intake per day compared to bulls offered the control supplement. Furthermore, elevated plasma urea nitrogen concentrations were observed in the bulls offered the hempseed meal. Bulls offered the hempseed meal performed similarly to those fed the same nutrient profile of standard, commercially available feed ingredients, indicating comparable nutrient availability. This demonstrates the potential of hempseed meal to effectively compete against readily available feed ingredients.

Abstract: Yearling rough-stock bulls (n = 38) were utilized in a randomized complete block design to evaluate dietary hempseed meal (HSM) inclusion on growth (ADG), intake (DMI), and efficiency (F:G). Bulls were blocked by body weight (BW), grouped into 10 pens (n = 3–4 bulls/pen), and randomly assigned to an HSM or control supplement treatment (CON; 72.5% cottonseed meal, 14.5% soy hulls, 13% fat). Treatments were offered at 10%, while 90% was fed as a mixed ration [50% Bermuda grass hay, 40% textured commercial feed (10% CP)]. Diet samples were dried and DMI was calculated. F:G was evaluated using DMI and ADG. Blood for plasma analysis and BW were obtained on sample days, prior to feed delivery. Data were analyzed using the GLIMMIX procedure in SAS version 9.4. The results were considered significant when $p \leq 0.050$. There was no treatment × time interaction, or treatment effect for interim BW, ADG, or F:G ($p \geq 0.100$). A treatment × time interaction occurred for DMI ($p < 0.01$), and BW ($p = 0.01$) increased in all bulls over time, while ADG decreased ($p = 0.005$), suggesting that interim live performance was not affected by HSM. Plasma urea nitrogen increased over time ($p < 0.001$) in all bulls, with greater concentrations observed in HSM bulls ($p = 0.043$).

Keywords: hempseed meal; performance; blood metabolites; urea nitrogen; growth; efficiency

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1. Introduction

Finding cost-competitive feed ingredients with sufficient nutritional profiles is important for a sustainable animal feed industry in North America. The human supplement industry is driving hemp oil production, resulting in by-products such as hempseed meal (HSM) [1]. Hempseed meal's nutritional content makes it a potential source of ruminant nutritional supplementation, particularly as a source of dietary fiber, fat, or protein. Hemp seed meal contains a mean crude protein (CP) content of 40.7% and a mean fat content of 10.2%, while the digestibility ranges from 90 to 97% [2]. Hessle and colleagues [3] compared soybean meal to cold-pressed hemp cake (HC) and found that feeding both products resulted in performance similarities for growing cattle and improved rumen function. This suggests that these by-products could compete with currently available feed ingredients. Furthermore, there is potential for increased sustainability if industrial hemp and its associated derivatives are utilized by producers. Hemp contributes to biodiversity within an environment, has a high carbon up-take, and does not require the use of pesticides and other chemicals to promote growth [4]. Hemp also has the potential to be a lucrative investment for the agricultural industry in the US. In a market report published by Expert Market Research [5], the global industrial hemp market has attained a value of over USD 5 billion in 2023 and is expected to grow around 19% from 2024 to 2032 [5,6]. Furthermore, in a market analysis conducted by Grand View Research [7], the global animal feed additives market had a value of approximately USD 40 billion in 2022 and is expected to grow at a rate of 3.5% from 2023 to 2030 [7]. In an article by Drotleff [1], it was stated that the executive director of the Hemp Feed Coalition, Hunter Buffington, reported that "one of the best ways to support farmers and livestock producers, is to commoditize the by-products that come out of producing and processing hemp". Processing hemp leads to the production of HSM and hemp cake as by-products. These by-products are valuable sources of nutrients, as demonstrated by the findings of the current study and the other literature, but large quantities are currently sitting idle rather than being utilized [1].

Current US legislation surrounding hemp includes the 2018 Hemp Farming Act. It legalized hemp growth and production in the US by licensed producers, so long as the plant materials do not exceed tetrahydrocannabinol (THC), specifically delta-9 THC, concentrations of 0.3% [8]. Any plant materials exceeding this limit are deemed to be marihuana, and therefore illegal under federal law [9]. This legislation allowed for the industrial hemp industry's expansion. According to a National Agricultural Statistics Survey conducted by the United States Department of Agriculture (USDA) in 2021, industrial hemp crops were valued annually at around USD 824 million [10]. Despite the potential of hemp derivatives as a feed ingredient, their use in animal feeds was not authorized via the 2018 Hemp Farming Act. This makes utilization by producers challenging. Organizations such as the American Association of Feed Control Officials (AAFCO) and the Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) are responsible for creating guidelines regarding the ingredients used in animal feeds [11]. There are also organizations, namely the Hemp Feed Coalition (HFC), whose goal is to gain federal approval for hemp and its derivatives as animal feed ingredients [12]. It was one of the first organizations to advocate for the use of HSM and HC as a protein supplement in poultry diets [13]. However, advocation for HSM supplementation in ruminant diets has not yet occurred.

Currently, AAFCO and the FDA are concerned with animal performance and safety, as well as possible cannabinoid deposition into meat, milk, or other constituents, as this could potentially impact consumer safety [14]. They have requested that more data regarding hemp's safety and efficacy be collected before its approval as an animal feed ingredient [15]. There have been some studies completed regarding the safety and efficacy of hemp derivatives as a feed ingredient in ruminant diets, including a study by Irawan et al. [16] where late-lactation dairy cows received a 13% pelleted spent hemp biomass (SHB). The researchers observed a decreased DMI in cows offered SHB, no effects on milk yield or components, a higher N use efficiency, and a potential decrease in liver clearance. Data obtained from this study led researchers to conclude that SHB was a safe feed ingredient for lactating dairy cows [16]. Parker et al. [17] investigated SHB's potential as an ingredient for ruminant diets by feeding it to finishing lambs at 10 and 20% of the diet. The researchers observed that none of the performance data evaluated, including liveweight gains, were different across the groups. Carcass and meat quality parameters also did not differ across feeding groups, apart from a larger carcass purge loss and meat cook loss in lambs fed 20% SHB. Also, in lambs fed 20% SHB, a decreased feed intake was observed primarily during the first period of this study. Despite this, there was an observed increase in intake in lambs fed 10% SHB that suggests a positive long-term effect of feeding SHB [17]. Hemp cake (HC) is another derivative of hemp that has been studied for use in ruminant rations. Arango et al. [18] evaluated the effect of dietary HC inclusion on veal calf performance, where no effect on final body weight, efficiency, intake, or average daily gain was observed. Calves offered the HC did, however, have a higher dressing percentage and carcass conformation. The researchers concluded that HC as a source of supplementation in a concentrate was safe due to the in vivo performance of the calves remaining largely unaffected [18]. Finally, Smith et al. [19] evaluated the excretion and residue depletion of cannabinoids in the liver, skeletal muscle, and other tissues from beef heifers offered HC at 20% for a total of 111 days. The researchers observed that neutral cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), were measured in adipose tissue at all withdrawal periods. Alternatively, cannabinoid acids, like cannabidiol acid (CBDA) and tetrahydrocannabinol acid (THCA), were observed to be depleted by the liver by withdrawal day 4. The researchers concluded that human exposure to cannabinoids through consumption of beef fat was a remote risk [19]. There have been few studies regarding the use of HSM, specifically, in ruminant diets.

As such, a knowledge gap exists regarding ruminant performance, particularly in cattle, when provided a diet supplemented with hemp derivatives. It is important to bridge this gap, particularly if we want to determine whether or not it is advantageous to supplement ruminant diets with these products. The objective of this study was to contribute to the current data regarding the safety and efficacy of dietary HSM as a potential feed ingredient and evaluate its impact on the performance of growing ruminants. This was performed by investigating the effects of dietary HSM supplementation on growth performance, feed intake, feed efficiency, and blood parameters in yearling rough-stock bulls.

2. Materials and Methods

2.1. Animal Care and Use

Animals were housed at the Tarleton State University Campus Agriculture Center, where this study was conducted from July to October 2022. The Institutional Animal Care and Use Committee (IACUC) of Tarleton State University approved all animal care and experimental procedures (IACUC Approval Number: 03-007-2022).

2.2. Experimental Design and Treatments

Yearling rough-stock bulls (n = 40) were utilized in a completely randomized block design. Bulls were blocked by body weight (BW; average initial BW = 261.13 kg $\pm$ 74.71 kg) and sorted into 10 pens (4 head/pen). Each pen was randomly assigned to one of two treatment groups (5 pens/treatment) of either a hempseed meal (HSM) supplement or a control (CON) protein supplement. Two animals were removed from this study due to circumstances unrelated to treatment, leaving one pen from each treatment with three head/pen and thirty-eight animals on trial. Bulls had ad libitum access to water. Feed and treatment delivery were recorded daily at each feeding time (0700 h and 1700 h). Orts, otherwise known as refusals, were also collected and documented daily prior to morning feed delivery. The refusals recorded were used to adjust the amount of feed given daily to be consistent with slick-bunk ad libitum feeding practices.

Bulls were subjected to a 28-d adjustment period prior to treatment initiation, where they were offered a basal diet of Bermuda grass hay (*Cynodon dactylon*) and a 10% crude protein (CP) textured feed (Stocker 10; Red Chain Feeds, Stephenville, TX, USA). Following the adjustment period, respective dietary treatments were provided for 63 d. This was a mixed ration consisting of 50% Bermuda grass hay, 40% Stocker 10, and 10% supplement. Supplements consisted of two treatments, HSM or CON. The CON supplement was formulated to have a similar macronutrient composition to the HSM supplement, and consisted of 72.5% cottonseed meal, 14.5% soybean hulls, and 13% fat/oil (Essentiom; Arm & Hammer

Animal Nutrition; Church & Dwight Co., Inc., Princeton, NJ, USA). Table 1 describes the composition of the basal diet and supplements offered, which were formulated to meet or exceed the nutrient requirements for growing beef cattle, and nutritional profiles were determined via wet chemistry analysis (Cumberland Valley Analytical Services; Waynesboro, PA, USA; NASEM, 2016).

Table 1. Nutritional composition of basal diet, control (CON), and hempseed meal (HSM) supplements fed to yearling rough-stock bulls [% dry matter (DM) basis].

		Supplement	
Ingredients, % DM	**Basal Diet**	**CON**	**HSM**
Bermuda grass hay	50.0		
Stocker 10	40.0		
Supplement	10.0		
Hempseed Meal	--	--	100.0
Cottonseed Meal	--	72.5	--
Soybean Hulls	--	14.5	--
Essentiom (fat/oil)	--	13.0	--
Nutritional Profile [3], DM			
DM, %	89.7	89.8	94.5
Crude Protein, %	10.5	34.4	34.8
Neutral Detergent Fiber, %	52.2	33.8	43.6
Crude Fat, %	3.0	14.2	16.1
Ash, %	5.8	8.2	8.9
Total Digestible Nutrients, %	66.2	80.8	68.9
NE_M [1], Mcal/kg	0.67	0.88	0.78
NE_G [2], Mcal/kg	0.40	0.58	0.50

[1] Net energy for maintenance, Mcal/kg; [2] Net energy for gain, Mcal/kg; [3] nutritional profiles were determined via wet chemistry analysis (Cumberland Valley Analytical Services; Waynesboro, PA, USA).

2.3. Sample Collection

Feed, treatment, and ort samples were collected daily and compiled over each week. At the end of the week, the samples were taken to the Tarleton State University Regional Dairy Center. There, the samples were dried in an oven (ThermoFisher Scientific Inc., Waltham, MA, USA) at 55 °C for a period of 48 h and later used to calculate DMI using the following equation:

$$\text{Feed Delivered (DM\%)} - \text{Orts (DM\%)}/\text{\# Bulls in Pen.} \quad (1)$$

ADG was also calculated using the following equation:

$$(\text{Empty Final BW} - \text{Empty Initial BW})/\text{\# of D in Feeding Period.} \quad (2)$$

Efficiency per period was then estimated based on calculated ADG and DMI values and was commonly referenced as the feed-to-gain ratio (F:G). On sample days 0, 21, 42, and 63 relative to treatment initiation, bulls were restrained in a manual squeeze chute, which was operated by trained personnel. Prior to collection of whole blood, BW was obtained and recorded by trained scribes. All measurements on sample days were obtained prior to daily feed delivery. Whole blood samples were collected via coccygeal venipuncture using an 18-gauge needle, vacuum tube hub, and two vacutainer tubes, one 10 mL non-additive red-top vacutainer tube and one 4 mL lithium heparin-added tube. Samples were then stored on ice until centrifugation.

2.4. Laboratory Analysis

Various blood metabolites were evaluated using collected whole blood samples. Sera were first separated from the whole blood sample by centrifugation at 1000× *g* for 20 min at 4 °C. It was then stored in quadruplicate 2 mL aliquots at −20 °C until subsequent analysis. Blood metabolite and plasma urea nitrogen (PUN) concentrations were quantified using the separated sera via colorimetric analysis using a VetScan Chemistry Analyzer (Abaxis Inc. and Zoetis, Parsippany, NJ, USA). In addition to PUN, blood metabolites quantified included proteins and enzymes such as albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), bilirubin (BIL), and creatinine (CRE). Also evaluated were essential electrolytes, including calcium (Ca), phosphorus (P), sodium (Na), and potassium (K). Amylase (AMY), glucose (GLU), total protein (TP), and globulins (GLOBs) were also evaluated.

2.5. Statistical Analysis

All data were analyzed as a randomized complete block design using the GLIMMIX procedure in SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA) with the pen as the experimental unit. The statistical model considered treatment as a fixed effect, while block was considered a random effect. Treatment and day were considered as main effects and evaluated with a covariate structure appropriate for repeated measures. A Kenward–Roger statement was included to account for necessary adjustments to degrees of freedom. Least squares means were generated using SAS LSMEANS statement. When a significant preliminary *F*-test was detected, data were separated and denoted as different using the pairwise comparison PDIFF and LINES options. Results were considered significant as determined by an α level of $\leq$0.050, with tendencies considered at $0.050 < p \leq 0.100$.

3. Results

3.1. Growth Performance and Feed Efficiency

There were no treatment × time interactions observed for body weight (BW; $p = 0.999$), average daily gain (ADG; $p = 0.563$), interim dry matter intake (DMI; $p = 0.672$), or efficiency (F:G; $p = 0.254$). Therefore, the overall performance of the animals offered the control (CON) and HSM supplements was not different. The main effects of treatment and time on these variables were evaluated. Interim DMI increased over time ($p = 0.007$), but when combined with ADG to determine F:G per period, the main effects were not found to be different ($p = 0.167$). There were also no effects of treatment observed for BW ($p = 0.996$), ADG ($p = 0.680$), interim DMI ($p = 0.608$), or F:G ($p = 0.341$). However, there was a tendency observed during the first interim period (d0 to d21) in which bulls offered the HSM supplement tended to gain less and be less efficient ($p = 0.080$; Table 2).

All bulls increased in BW over time ($p = 0.001$), while a decreased ADG was observed ($p = 0.005$), but not as a result of treatment. Furthermore, there was a treatment × time interaction for daily DMI. It was observed that bulls offered HSM were able to maintain an elevated DMI per day compared to CON bulls ($p < 0.001$). Bulls offered the CON supplement were observed to have a lower intake in the beginning of this study, and it increased over time, as seen in Figure 1.

Table 2. Effects of hempseed meal (HSM) supplementation versus control (CON) supplement on interim, per-period rough-stock bull performance, including initial and final weight (kg), average daily gain (ADG), dry matter intake (DMI), and efficiency (F:G).

	Treatment [1]			*p*-Value [3]		
Item	**CON**	**HSM**	**SEM [2]**	**TRT**	**Day**	**TRT × Day**
Initial Weight, kg	258.83	263.43	74.71	0.970	-	-
Final Weight, kg	320.40	324.69	66.84	0.957	-	-
Total Performance						
d 0–63						
ADG, kg	1.01	0.97	0.19	0.683	<0.001	0.563
DMI, kg	7.25	8.38	1.01	0.103	<0.001	<0.001
F:G, kg/kg	8.08	9.75	4.02	0.341	0.167	0.254
Interim Performance						
d 0–21						
ADG, kg	1.12	0.88	0.23	0.076		
DMI, kg	6.56	8.31	1.25	0.441		
F:G, kg/kg	5.96	11.74	7.32	0.084		
d 21–42						
ADG, kg	1.18	1.19	0.21	0.974		
DMI, kg	7.32	8.36	0.98	0.651		
F:G, kg/kg	6.32	7.32	1.96	0.701		
d 42–63						
ADG, kg	0.73	0.84	0.13	0.401		
DMI, kg	7.87	8.49	0.82	0.868		
F:G, kg/kg	11.97	10.18	2.79	0.581		

[1] CON = 10% of diet control supplement; HSM = 10% of diet hempseed meal; [2] standard error of the mean; [3] *p*-values are presented for treatment time × interactions and main effects for total performance and the main effect of treatment at specific time points for interim performance.

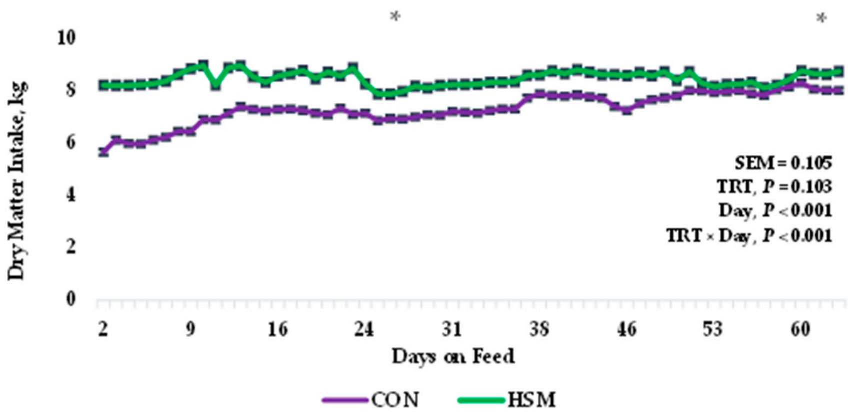

Figure 1. Dry matter intake (DMI) per day of control (CON) and hempseed meal (HSM) diets. The *x*-axis describes days on feed, and the *y*-axis describes DMI/kg. The purple line depicts CON, while the green line depicts HSM. There was a treatment × time interaction observed regarding daily DMI, where HSM bulls maintained an elevated DMI throughout this study, while CON DMI increased gradually over time ($p < 0.001$). Asterisks (*) above the bars indicate significant differences ($p \leq 0.050$).

3.2. Plasma Urea Nitrogen

There was no treatment × time interaction observed for plasma urea nitrogen (PUN) concentrations ($p = 0.170$). There was a day effect observed on PUN concentrations, where it was observed that average concentrations increased over time, though not as a direct result of treatment ($p < 0.001$; Figure 2). Furthermore, there was a treatment effect observed on PUN, in which bulls offered the HSM supplement often had greater average PUN concentrations in comparison to the bulls offered the CON supplement ($p = 0.043$).

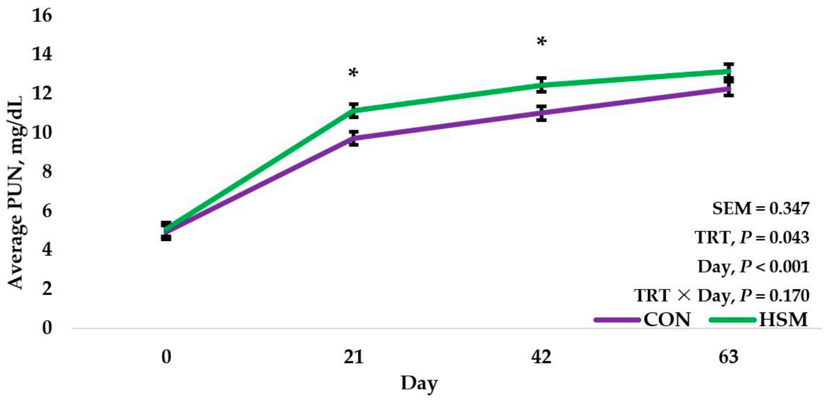

Figure 2. Average plasma urea nitrogen (PUN) concentrations. The *y*-axis represents the average PUN concentration in mg/dL. Sample d0, d21, d42, and d63 are on the *x*-axis. Depicted in this figure is a day effect in which average PUN concentrations increased over time, from d0 to d63 ($p < 0.001$). Treatments, CON and HSM, are represented by the purple and green lines, respectively. Depicted in this figure is a treatment effect in which average PUN concentrations were greater in the bulls offered the HSM supplement, compared to the bulls offered the CON supplement ($p = 0.043$). Asterisks (*) above the bars indicate significant differences ($p \leq 0.050$).

3.3. Other Blood Parameters

There were no treatment × time effects observed for alkaline phosphatase (ALKP), alanine aminotransferase (ALT), amylase (AMY), bilirubin (BIL), calcium (Ca), phosphorus (P), creatinine (CRE), or sodium (Na) concentrations ($p \geq 0.100$). Treatment, however, did tend to affect albumin (ALB) and glucose (GLU), where concentrations were lower in bulls offered the HSM supplement ($p = 0.065$). There were also day effects observed where ALB concentrations tended to decrease, then be maintained over time ($p = 0.054$), while ALT concentrations increased over time ($p = 0.017$) in all animals. Day effects were also observed for AMY and Ca, where concentrations increased during the first interim period (d0 to d21), then slightly decreased, and were maintained throughout the remaining periods, from d21 to d63 ($p = 0.002$). The effects of HSM supplementation on blood parameters are summarized in Table 3.

Table 3. Effects of hempseed meal (HSM) supplementation on average rough-stock bull blood parameters on sample d0, d21, d42, and d63.

	CON [1]				HSM [1]				*p*-Values		
	D0	D21	D42	D63	D0	D21	D42	D63	TRT	Day	TRT × Day
Albumin, g/dL	2.93 ^{a}	2.53 ^{b}	2.6 ^{b}	2.71 ^{ab}	2.58 ^{a}	2.44 ^{b}	2.5 ^{b}	2.58 ^{ab}	0.065	0.054	0.613
Alkaline phosphate, U/L	169.13	145.22	145	131.68	120.68	150.93	128	130.47	0.25	0.823	0.415
Alanine aminotransferase, U/L	22.53 ^{b}	26.33 ^{ab}	27.29 ^{a}	28.79 ^{a}	24.93 ^{b}	26.93 ^{ab}	28.89 ^{a}	31.05 ^{a}	0.148	0.017	0.811
Bilirubin, mg/dL	0.74	0.26	0.26	0.24	0.18	0.26	0.23	0.22	0.299	0.595	0.44
Creatine, mg/dL	15.9	1.18	1.24	1.35	1.47	1.09	1.18	1.2	0.323	0.408	0.43
Calcium, mg/dL	9.32	10.06	9.82	9.93	8.18	10.49	9.74	9.87	0.478	0.002	0.131
Phosphorus, mg/dL	8.07	7.28	8.26	7.27	7.64	7.61	8.2	7.97	0.356	0.147	0.774
Sodium, mmol/L	137.8	138.33	139.07	141.31	135.13	137.93	137.78	139.53	0.617	0.955	0.99
Potassium, mmol/L	6.13 ^{a}	4.92 ^{b}	4.77 ^{bc}	5.01 ^{b}	4.5 ^{c}	5.19 ^{b}	4.94 ^{b}	5.27 ^{b}	0.184	0.642	<0.001
Amylase, U/L	23.87	42.06	40.43	44.89	36.25	57.47	63.89	45	0.297	0.002	0.45
Glucose, mg/dL	147.2	135.94	128.64	131.53	121.8	11.53	98.11	114.05	0.065	0.173	0.798
Plasma urea nitrogen, mg/dL	4.92	9.72	11	12.26	5.06	11.13	12.44	13.15	0.043	<0.001	0.17
Globulin, g/dL	3.66	4.63	4.64	4.83	4.67	5.32	5.2	5.08	0.025	0.03	0.894
Total protein, g/dL	7.81 ^{a}	7.34 ^{bc}	7.21 ^{c}	7.53 ^{bc}	7.71 ^{abc}	7.77 ^{ab}	7.71 ^{ab}	7.68 ^{abc}	0.196	0.254	0.027

[1] CON = 10% of diet control supplement; HSM = 10% of diet hempseed meal. ^{abc} Averages within the same row with different superscripts are statistically different.

There was a treatment × time effect observed for potassium (K) and total protein (TP). For K concentrations, it was observed that levels were very different during the beginning of this study, primarily during the first interim period (d0 to d21). As this study progressed, observed K levels became more similar despite treatments ($p < 0.001$), which can be seen in Figure 3.

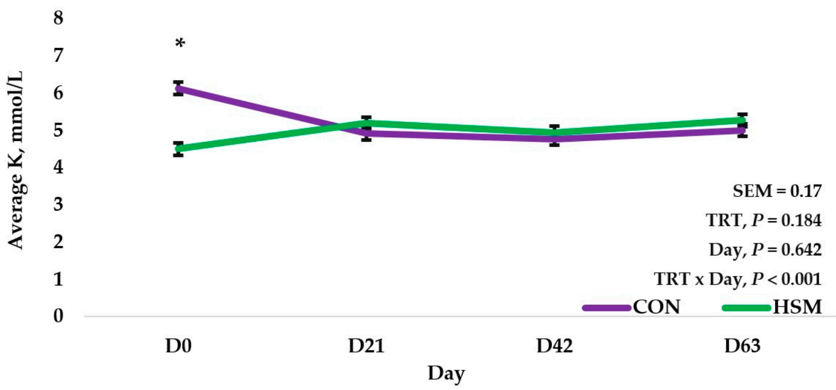

Figure 3. Potassium (K) levels per sample day for the bulls on the CON and HSM diets. On the *x*-axis are sample days (d0, d21, d42, and d63), and on the *y*-axis are the average K levels in mmol/L. The purple line depicts the CON treatment, and the green line depicts the HSM treatment. There was a treatment × time effect observed for K, where levels started out different between the two treatments during the first interim period (d0–d21), and as the trial progressed, bulls ended with similar K levels despite the diets ($p < 0.001$). Asterisks (*) above the bars indicate significant differences ($p \leq 0.050$).

There were main effects of both treatment and day on globulin (GLOB) concentrations. In terms of a treatment effect, bulls offered the HSM supplement had greater average GLOB concentrations than bulls offered CON ($p = 0.025$; Figure 4). Furthermore, GLOB concentrations were observed to increase over time, though not as a direct result of the treatments offered ($p = 0.030$). This likely led to the trend in average TP concentrations, which changed over time and were lower for bulls offered the CON supplement; meanwhile, bulls offered HSM were observed to maintain a slightly elevated concentration throughout this study ($p = 0.027$; Figure 5).

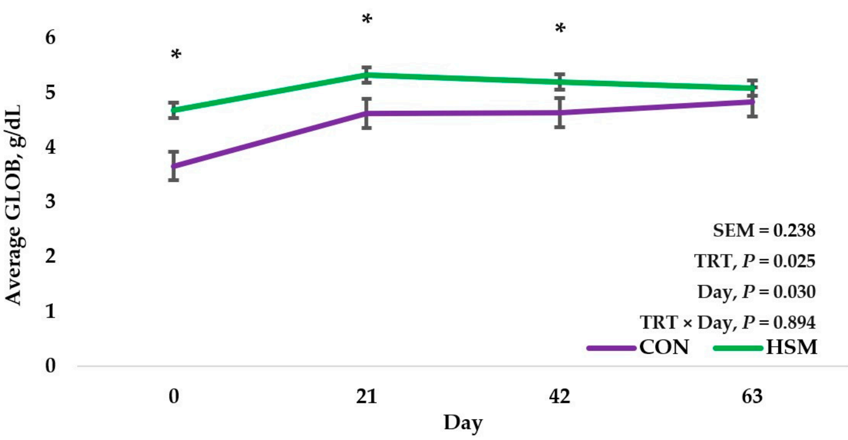

Figure 4. Average globulin (GLOB) concentrations over time. The *y*-axis represents the average GLOB concentrations in g/dL. Sample days (d0, d21, d42 and d63) are on the *x*-axis. Treatments, CON and HSM, are represented by purple and green lines, respectively. Depicted here is a day effect in which average GLOB concentrations increased over time, from d0 to d63 ($p = 0.030$). A treatment effect in which average GLOB concentrations were greater in the bulls offered the HSM supplement, compared to those offered the CON supplement ($p = 0.025$) is also displayed. Asterisks (*) above the bars indicate significant differences ($p \leq 0.050$).

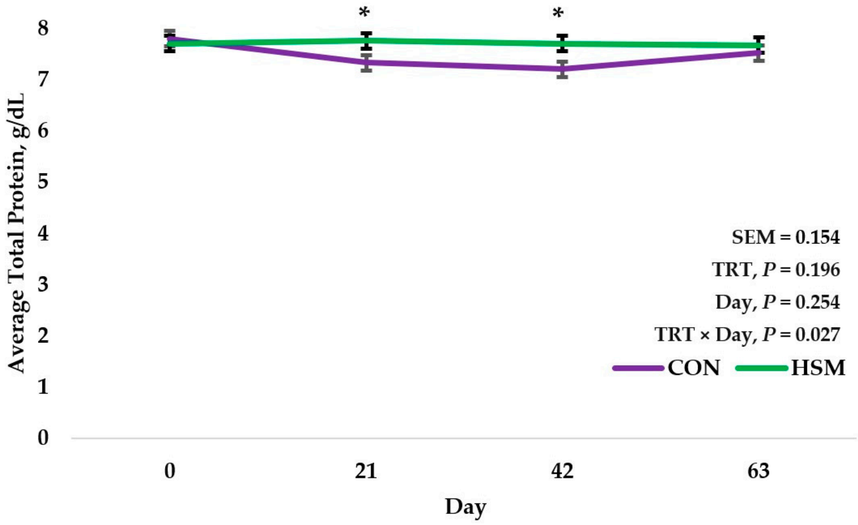

Figure 5. Average total protein concentrations over time. The y-axis represents the average total protein concentrations in g/dL. Sample days (d0, d21, d42, and d63) are on the x-axis. Treatments, CON and HSM, are represented by purple and green lines, respectively. Depicted here is a treatment by time interaction for total protein, where treatments differed on d 21 and 42 (p = 0.027). Asterisks (*) above the bars indicate significant differences ($p \leq 0.050$).

4. Discussion

By investigating the effects of dietary hempseed meal (HSM) supplementation on growth performance, feed intake, feed efficiency, and blood parameters in these bulls, insight was provided on the effect that this supplementation may have on growing ruminants. The lack of statistically significant effects of HSM supplementation on animal performance, including average daily gain, body weight, and interim dry matter intake, suggested that interim live performance of animals was not affected by HSM. This has also been reported in other studies [20,21]. The treatments used, control (CON) and HSM supplements, were formulated to have similar macronutrient compositions. While they were similar in several aspects, there were a few notable differences in nutrient composition between treatments. As can be seen in Table 1, the HSM supplement had a slightly greater CP content of 34.8%, while the CON supplement had a CP content of 34.4%. The HSM supplement also had a greater neutral detergent fiber (NDF) and crude fat (CF) content of 43.6% and 16.1%, respectively, compared to the 33.8% and 14.2% of the CON supplement. While the NDF content was comparable, the CF content of the HSM used in this study was greater than the levels reported in other studies, like the one conducted by House et al. [2], where 10 cold-pressed HSM samples were evaluated for protein quality. They determined that the average CP content for hempseed meal was 40.7%, the average NDF content was 30.5%, and the mean CF content was 10.2% [2]. The elevated CF content of the HSM used in this study was a result of processing variation. The similarity in nutrient composition between treatments explains why few differences were observed regarding rough-stock bull performance. The similarity in nutrient composition also suggests a similar total digestible nutrient (TDN) content between supplements, though it was not evaluated in this study. A more in-depth chemical analysis of the diets is warranted, particularly to determine potential differences in digestibility between the treatments.

Addo et al. [20] observed that feeding hempseed meal instead of canola meal to lactating dairy cows did not affect their dry matter intake (DMI), which correlates with the observation made in this study that interim DMI was not influenced by HSM. Additionally, Winders et al. [21] evaluated the effect of hemp cake (HC) inclusion at 20% of the diet in finishing heifers. Heifers fed hemp diets had lower final body weight and efficiency than heifers offered dried distillers' grains (DDGS). This correlates with the current study's findings, where bulls offered HSM at 10% tended to gain less efficiently, during the first interim period, d0–d21, indicating a delayed effect of nutrient retention. Additionally, the

HC provided at 20% of the diet did not influence DMI [21]. The interim DMI of the bulls in the current study increased over time, though not as a direct result of treatment. There are many factors that can influence the DMI of cattle, including the animal itself, the feed ration provided, and the environment of the animal [22]. There was an increase in body weight of the bulls throughout this study, and it is inferred that the increased interim DMI of the bulls in this study is attributed to this weight gain. There was also an associated decrease in average daily gain of all the bulls in this study over time. Thus, while the bulls were still gaining weight, the margin of gain decreased.

Bulls offered HSM experienced elevated plasma urea nitrogen (PUN) concentrations compared to CON bulls. Despite this increase in PUN, urea nitrogen levels remained within normal limits for cattle, which are reported to be anywhere from 10 to 20 mg/dL [23]. The values in HSM bulls ranged from 5 to 13 mg/dL. These findings are consistent with other studies [21–25]. Winders et al. [21] also observed elevated plasma urea nitrogen in heifers offered HC. In another study by Winders et al. [24], the effects of HC inclusion on ruminal fermentation, organic matter intake, total track digestion, and nitrogen balance were evaluated in steers. The researchers observed that steers offered the hemp diet had superior nitrogen retention and ruminal and total tract nitrogen digestibility [24]. A similar effect was observed in growing meat goats, where blood urea nitrogen concentrations increased linearly with increasing levels of HSM [25]. The elevated PUN concentrations observed could be attributed to several factors, such as the elevated intake observed for the HSM bulls or the fact that the amount of protein in the diet was not adjusted as the bulls grew. Winders et al. [21] hypothesized that the increase in urea nitrogen concentrations they observed was attributed to an elevated CP and excess metabolizable protein. These factors can influence digestibility and lead to increased urea production [21]. Furthermore, there were decreased TP and PUN concentrations observed in the CON bulls compared to the HSM. These decreased concentrations may indicate that the CON bulls are more efficiently utilizing the protein in the diet compared to the HSM bulls. However, this could also correlate with the idea that there may be differences in nitrogen metabolism between the two treatments offered. This is something that warrants further investigation.

In the study conducted by Winders et al. [21], plasma glucose (GLU) concentrations were evaluated in finishing steers. They observed that GLU concentrations were not different between treatments; however, there was a day effect reported in which concentrations increased for a period before plateauing [21]. This differs from the findings of the current study, where bulls offered the HSM supplement tended to have lower plasma GLU concentrations compared to the CON supplement bulls. There was an interesting study conducted by Mollard et al. [26], where the effects of hemp protein consumption on glycemic control were investigated. In Mollard's study, 40 g and 20 g of hemp protein were compared to the same doses of soybean protein in two experiments. Treatments reduced blood GLU levels in both experiments, thus suggesting that hemp protein led to lower post-prandial blood GLU and insulin concentrations [26]. This could suggest that HSM is potentially linked to glycemic regulation but is also likely dose-dependent. It is important to note, however, that elevated levels of protein, fat, and fiber can lower glucose levels by slowing the rate of digestion [27]. As mentioned earlier in the discussion, the HSM supplement used in the current study had an NDF and CF of 43.6% and 16.1%, respectively (Table 1). Neutral detergent fiber (NDF) is a measure of fiber in the diet, particularly of plant cell structural components, and contributes to fill and the digestibility of a feedstuff [28]. A greater NDF content, in addition to contributing to filling the gut, is also linked to a slower rate of digestion. Based on this knowledge and information from other studies in the literature, the elevated CF and NDF of the HSM supplement used in the current study is most likely what contributed to the tendency for decreased plasma GLU concentrations observed in the HSM bulls.

There was little information available from other literature sources regarding the other statistically significant metabolites mentioned: potassium (K), total protein (TP), and globulins (GLOBs). Potassium (K) serves as a means of maintaining fluid homeostasis within the cell. There are several factors that influence K concentrations, including insulin. Insulin and catecholamines assist in the shifting of K into the cell. If there is not enough of either present, it could lead to an increase in extracellular K [29]. As mentioned previously, the study conducted by Mollard et al. [26] found that hemp protein lowers blood GLU and insulin levels. Additionally, intake can also influence extracellular K balance. In a species with elevated K in the diet, like ruminants, changes in intake alone can influence plasma K concentrations [30]. Therefore, with an increasing level of intake, there would be a mild increase in the excretion of extracellular K. As a result, there would be an increase in blood K levels associated with an increased intake. In the current study, it was observed that bulls offered the HSM supplement were able to maintain an elevated daily DMI over time. This likely contributed to the fluctuation observed in plasma K concentrations.

Abrahamsen et al. [25] evaluated the effect of HSM supplementation on the plasma chemistry of growing meat goats and reported a lower plasma TP concentration. This conflicts with the findings of the current study, in which the bulls offered HSM were observed to have greater TP concentrations over time. The concentrations fell within normal limits, however, which are reported to be 6.7 to 7.5 g/dL [23]. Elevated TP levels could be attributed to the HSM supplement offered. However, bulls offered HSM in the current study were observed to have increased concentrations of GLOB, which is likely to be what contributed to the elevated TP concentrations and could also be an indication of antigenic stimulation in the HSM bulls.

Globulins (GLOBs) consist of non-albumin proteins in the body, particularly those produced in response to inflammatory stimuli [31]. Despite observing elevated GLOB concentrations in the bulls offered the HSM supplement, concentrations fell within the normal range for cattle, which is reported to be 2.8 to 5.4 g/dL [32]. Increases in GLOB concentrations typically result from an increase in immunoglobulins or other proteins [33]. Albumin (ALB) makes up most of the total protein in the plasma, while the rest is made up of total globulins. Its functions include the regulation of osmotic pressure, transportation of hormones, and more [33]. It is important to note that blood protein levels can be altered due to the animal being in a fasting state. Blood samples collected during this study were taken prior to morning feeding, thus contributing to the tendency for slightly decreased ALB levels as observed. The concentrations of ALB fell just below the normal range for cattle, which is reported to be 3.3 to 4.3 g/dL [32].

5. Conclusions

Industrial hemp by-products, particularly hempseed meal (HSM), still need approval before their implementation as animal feed ingredients. Prior to this approval, governing organizations have requested more data be presented regarding the safety and efficacy of these products. The similarity in performance observed between the two treatments, as seen by the lack of significant effects of 10% dietary HSM supplementation on interim live performance, suggests that the nutritional availability between the two supplements is comparable. Due to the elevated dry matter intake and increased plasma urea nitrogen and total protein concentrations observed in bulls offered the HSM supplement, further investigation regarding nitrogen metabolism between the different treatments is warranted. Bulls offered HSM also experienced elevated globulin concentrations, while other blood metabolites evaluated remained largely unaffected by treatment. As demonstrated by the findings of the current study, HSM can compete effectively against readily available feed ingredients, particularly cottonseed meal and soybean hulls, which are common sources of protein supplementation used in ruminant rations.

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