Journal of Agricultural Science and Technology (JAST), 28(3)
In Press, Pre-Proof Version

Evaluating raw and heat-treated hempseed (*Cannabis sativa* L) with enzyme supplementation for broiler chicken on growth, digestibility, morphometric and gut microbiota

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ABSTARCT

A total of 480 seven-day-old male Arian broiler chickens were divided into five treatment groups with six replicates each. The treatments were offered to the birds for three weeks (days 7 to 28) and included a control group, 10% raw hempseed (*Cannabis sativa* L) supplementation (RH), 10% RH with enzyme addition (RHE), 10% heat-treated hempseed (HH) in the diet, and 10% HH with enzyme supplementation (HHE). A completely randomized design with a 2 × 2 factorial arrangement (raw vs. heat-treated hempseed and with vs. without enzyme supplementation), plus a control group, was used. While dietary treatments (hemp supplementation) significantly increased body weight and feed intake, the heat processing decreased weight gain. Hemp supplementation significantly lowered Coliform and increased Lactobacillus content in the ileum, while processing increased Lactobacillus and enzyme addition decreased *E Coli* ($P < 0.05$). Digestibility parameters were positively affected by enzyme addition ($P < 0.05$) but protein digestibility was reduced by heating. There were no significant interaction effects (enzyme x supplementation and heat treatment) except for the Total Aerobes count of intestinal micro flora ($P < 0.05$). In conclusion, hempseed addition in the diet of broiler chickens during 7-28 days of age improved broiler performance and enzyme supplementation improved microbiology and more profoundly digestibility parameters.

Keywords: Hempseed, heated hempseed, enzyme, broiler.

INTRODUCTION

Hempseed (*Cannabis sativa* L) along with its by-products such as hempseed oil and meal, show potential as feed ingredient for livestock. It contains about 25% crude protein, 33-

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35% oil, 34% carbohydrates (mostly as fiber), and 18.3 MJ/kg (4308 kcal/kg) of metabolizable energy, and is rich in essential minerals and vitamins. Its primary protein, edestin (a *highly-digestible, hexameric legumin protein)*, is particularly noted for its high essential amino acid content (Gakhar et al., 2012; Wang et al., 2008).

Historically, hemp cultivation was restricted in many countries until the early 2000s. Although industrial hempseed (with less than 0.3% tetrahydrocannabinol) is now approved for human consumption, its use in animal feed was once deemed "unsafe." Recent research, however, has begun to explore its potential benefits as hempseed has become more widely legalized (Shariatmadari, 2023).

Early studies on hempseed's impact on broiler performance revealed varied outcomes. For instance, Khan et al. (2010) reported that including 10% hempseed significantly improved body weight and feed efficiency. Mahmoudi et al. (2015) noted that while 2.5% hempseed had no effect, 7.5% was optimal for weight gain. Skrivan et al. (2020) found no effect with 4% hempseed but noted improved tibia bone strength. Parr et al. (2020) observed that a 20% hemp heart led to increased weight gain and better feed efficiency compared to a soybean meal-based diet. These studies support the safety of up to 10% hempseed inclusion, despite the presence of some antinutritional factors such as trypsin inhibitors, fiber, condensed tannins, phytic acid, and saponins (Russo and Reggiani, 2013).

To address these anti nutritional factors, heating and exogenous carbohydrase enzyme supplementations have been proposed as strategies to improve hempseed's effectiveness. Konca et al. (2019) demonstrated enhanced performance and egg quality in layer chickens fed 15% heat-treated hempseed as compared to similar amount of row hemp seed. As there was no report on effect of exogenous enzyme supplementation, an enzyme cocktail containing two main commonly supplemented carbohydraze (glucanaze and xylanase) was hypothesized to enhance chicken performances (Monyaka et al 2016: Mathlouthi,et al 2002). The combined effects of heat-treated hempseed and enzyme supplementation were also considered (Amerah et al 2011). Thus, the aim of this study was to evaluate the combined effects of heat-treated hempseed and an exogenous enzyme cocktail on broiler performance, ileum nutrient digestibility, and microbiota composition at a 10% dietary inclusion level. As young chicks have a less developed digestive tract, they are unable to produce enzyme in sufficient quantities by themselves and may not tolerate high fibreous diet. According to Wang et al (2017) chicks benefit more from enzyme addition at a younger age. Therefore, this experiment was designed to assess performance criteria up to 28 days.

MATERIALS AND METHODS

Diets, Birds and Housing

Samples of hemp seed (table 1) and experimental diets (table 2) were chemically analyzed in duplicate according to standard methods of the Association of Official Analytical Chemists (AOAC, 2005) for dry matter (at 105°C overnight), ash (oven at 600 overnight), crude protein (N x 6.25 - Kjeldhal), crude fat (Soxhlet extraction) gross energy by bomb calorimetric (Gallenkamp Autobomb, UK) and crude fiber. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured according to the procedures of Van Soest et al. (1991) and Robertson and Van Soest (1981), respectively. The method used for AA profiling was based on the standard protocol of the Pico-Tag method from Waters Corporation. High-performance liquid chromatography (Waters, Model: 2695E, USA) was used to determine samples following hydrolysis by hydrochloric acid (6 N) and derivatization by orthophaldialdehyde. Metabolisable energy was estimated according to Klis and Fledderus (2007). Other nutrient compositions are calculated based on NRC (1994) data of feedstuffs nutrient tables.

A total of 480 one-day-old male Arian broiler chickens were randomly divided into five treatment groups, with each group housed in six replicate pens containing 16 chickens each. The treatment groups were:

- Control (no hempseed)
- 10% raw hempseed (RH) in the diet
- 10% RH with enzyme supplementation (RHE)

- 10% heat-treated hempseed (HH) in the diet
- 10% HH with enzyme supplementation (HHE)

The birds were given a starter diet from days 0 to 6, and then were weighed randomly divided and switched to the experimental diets from days 7 to 28. Hempseed underwent to heat treatment at 120°C for 60 minutes, following the method as described by Konca et al. (2019). Two NSPase enzymes, Econase XT (endo-1,4-β-xylanase, with a minimum activity of 4,000,000 BXU/g) and Econase GT 200 (endo-1,3(4)-β-glucanase, with a minimum activity of 200,000 BU/g), were obtained from AB Vista, in the Netherlands. The enzyme treatments consisted of a mixture of 4 g/ton of Econase XT and 100 g/ton of Econase GT 200.

All birds had free access to feed and water throughout the experiment. The diet's composition and nutrient content are outlined in Table 1 and were formulated based on the Arian breeding guide (Corporation Support of Animal Affairs, 2008). Arian is a descendant of the Hybro Normal breed, originally developed by the Dutch company and now widely bred in Iran.

The initial temperature in the house was set to 33 ± 1°C for the first week and then gradually lowered to 24 ± 1°C for the subsequent weeks. The humidity level was consistently maintained at 60% throughout the study. The lighting schedule began with 23 hours of light and 1 hour of darkness from days 1 to 3, increasing by 2 hours of light each day until a final schedule of 16 hours of light and 8 hours of darkness was established, which continued for the remainder of the experiment. Light intensity ranged from 3–4 lux during the first week and increased to 5–7 lux thereafter. Each pen, measuring 1 m × 2 m, had a stocking density of less than 25 kg/m² and was fitted with a nipple drinker and feeder to ensure continuous access to food and water for the birds. Regular cleaning was performed in the broiler room to maintain hygiene standards throughout the experiment.

Measurements and Sampling

During the experimental period from days 7 to 28, we measured performance metrics including feed intake (FI), body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR). We tracked daily mortality to adjust FI, live weight, and FCR calculations. At the conclusion of the study, we randomly selected 12 birds from each treatment group, with two birds chosen from each replicate pen. After a 2-hour fasting period with water access, the birds were killed by cervical dislocation and exsanguination. The carcasses were plucked, and samples were taken for analysis of carcass characteristics, ileum content (to evaluate digestibility,

At 28 (end of experiment) two birds from each replicate pen were slaughtered to evaluate intestinal bacterial populations. The ileum (from Meckel's diverticulum to 5 cm before the ileocecal colonic junction) of each bird was cut open to collect approximately one gram of mixed and homogenized digesta. To determine the Colony Forming Units (CFU), the drop count method was used in saline solution (Miles and Misra, 1938). Each sample of ileal contents were homogenized, and then 1g of each sample was collected and transferred into 9 ml sterile saline solution to prepare serial dilutions. Plate count agar (Merck, Darmstadt, Germany), MacConkey agar (Himedia laboratories, Mumbai, India) and MRS agar (Merck, Darmstadt, Germany) were used for enumeration of total aerobes, *Escherichia coli* and lactic acid bacteria, respectively, following 24-hour aerobic incubation at 37°C (Jabbar et al., 2024).

For digestibility trial, the diet was top-dressed with 3g Marker (Titanium dioxide)/kg in last 4 days of experiment. Frozen ileal contents were thawed and dried at 60°C using a hot-air oven. Similar methods as for dietary component analysis (above) were applied for these samples. Apparent ileal digestibility for nutrients and energy was calculated following the methods described by Del Alamo et al. (2008) and Latifi et al. (2023).

Statistical Analysis

A completely randomized design with a 2 × 2 factorial arrangement (raw vs. heat-treated hempseed and with vs. without enzyme supplementation), plus a control group, was used. Data were analyzed using a two-way ANOVA through the GLM procedure in SAS (SAS, 2020) for the factorial part. Additionally, a one-way ANOVA was performed to compare the control group with all other treated diets. A significance level of $P < 0.05$ was applied, and significant differences were identified using Tukey's test.

RESULTS AND DISCUSSION

The composition of hempseed analyzed in this study (Table 1) was consistent with previous reports by Callaway (2004) and House et al. (2010). While hempseed and its by-products have been utilized for medicinal purposes for centuries (Della Rocca et al., 2020), there remains a considerable lack of understanding regarding their nutritional value and impact on poultry performance.

The effects of 10% RHS and HHS with and without multi-enzyme supplementation (G and X) on broiler chicken performance from 7 to 28 days of age has been evaluated. The initial average live weight of day-old broiler chickens was 41.0±1.7 g, increasing to approximately 153±4.4 g by 7 days of age. The performance metrics of chickens during the experiment are presented in Table 3. Chickens fed a diet supplemented with raw hempseed had a significantly higher ($P < 0.05$) body weight at 28 days of age compared to those fed the control diet. No additional benefit of enzyme addition or heat treatment was observed in this group. The pattern for feed intake was similar to body weight gain, with no significant effect of treatment (heating and enzyme addition) on feed efficiency ratio. Mortality was only observed in the control group, and hempseed supplementation did not affect livability.

The existing research on the effects of hempseed supplementation is relatively sparse, which complicates detailed comparative assessments. The initial scientific investigation into hempseed's impact on poultry (layer chicken) was conducted by Silversides and Lefrançois (2005). The earliest study specifically examining the influence of hempseed on broiler performance was conducted by Khan et al. (2010). According to a review by Shariatmadari (2023), there are only a limited number of studies that directly explore the effects of hempseed on broiler performance.

While hempseed and its by-products have been utilized for medicinal purposes for centuries (Della Rocca et al., 2020), there remains a considerable lack of understanding regarding their nutritional value and impact on poultry performance. The literature shows varying results regarding the impact of hempseed on feed intake. Mahmoodi et al. (2015) and Bahar et al. (2014) reported no significant change in feed intake with hempseed supplementation. In contrast, Skrivan et al. (2020) observed an increase in feed intake among broilers consuming hempseed. However, Khan et al. (2010) found that hempseed-fed broilers had reduced feed intake. Some believe that hempseed's tetrahydrocannabinol (THC) content may stimulate appetite and feed intake, impacting eating behavior and body weight regulation (Mahmoodi et al. 2015). However, at high inclusion levels (20%), elevated THC levels can have adverse effects on appetite and body weight (Vispute et al. 2019).

High hempseed inclusion may depress feed intake due to its high crude ash (8.8%) and cellulose content (House et al., 2010), which can be particularly problematic for younger birds. Vispute et al. (2019) reported reduced feed intake and body weight gain in early life stages, likely due to less developed gut mucosa and digestive enzymes. Konca et al. (2019) attributed lower feed intake to the characteristic flavor of raw hempseed, with heating enhancing flavor and increasing feed intake.

Regarding enzyme supplementation, Doskoviv et al. (2013) found no impact on feed intake, while Francesch et al (2009) suggested enzymes might decrease feed intake by increasing energy availability. Alternatively, enzymes could increase feed intake by reducing digestive content viscosity, enhancing nutrient digestibility (Lázaro et al., 2004: Wiśniewska et al., 2023).

The observed improvement in performance with hempseed indicates its nutritive value. Hempseed is recognized for its excellent protein quality and amino acid profile (Callaway, 2004), along with beneficial fatty acids, vitamins, and minerals, contributing to better performance. However, Konca et al. (2014) suggested that excessive amino acids from hempseed might imbalance amino acid ratios, reducing bioavailability. Roasting and enzyme supplementation mitigated some negative effects of hempseed inclusion.

All birds, except those in the control group (8% mortality), remained healthy throughout the experiment. Potential health benefits of hempseed may be due to orexigenic, anti-inflammatory, antipyretic, and antiparasitic effects of tetrahydrocannabinol (Callaway 2004; Mechoulam and Hanu, 2001). Cannabis sativa is reported to alleviate stress, improve immunity, and exhibit antimicrobial and antiviral properties (Novak et al., 2001; Sakakibara et al., 1991).

Microflora of the ileum

Dietary treatments while reducing Coliform content, increased Lactobacillus content of the ileum ($P < 0.05$) due to dietary hempseed inclusion (Table 4) Total aerobes were not influenced by raw hempseed inclusion but were reduced with enzyme addition and heat-treated hempseed diets ($P < 0.05$). Heat-treated hempseed significantly increased *Lactobacillus* while enzyme inclusion reduced Coliform counts ($P < 0.05$). There was significant ($P < 0.05$) enzyme and heating interaction effect on total aerobes counts.

The poultry industry faces challenges from pathogenic diseases, impacting mortality and production. Microbial content in the digestive tract plays a crucial role in gut health (Markovi et al., 2009). Industrial hempseed contains essential oils and cannabinoids that inhibit microbial growth (Nissen et al., 2010). However, Stastnik et al. (2016) found that higher cannabidiol levels did not affect microbiological parameters in the ileum. Conversely, Vispute et al. (2019) reported decreased Coliform counts and increased *Lactobacillus* counts in the caecum and jejunum with hempseed supplementation. Enzyme supplementation in our study reduced Coliforms and heating increased Lactobacillus counts. Bedford and Cowieson (2012) noted that exogenous enzymes can influence nutrient partitioning and bacterial populations, though effectiveness varies based on several factors such as the strain, age, health status/disease challenge of the animals, presence of antibiotics, quality of ingredients fed, along with the type (and levels) of enzyme employed.

Ileal digestibility

No general increase in digestibility parameters was observed with RHS inclusion (Table 5). Digestibility was largely unaffected by treatment groups, except for enzyme supplementation. Heat treatment lowers anti-nutritional compounds, increases protein availability, and enhances enzyme susceptibility (Maesman et al., 1995). Overheating can damage heat-sensitive amino acids and reduce the bioavailability of some minerals and vitamins (Harrel, 1990). Although heating did not affect digestibility, the heated group showed increased weight gain, likely due to higher feed intake. Previous studies suggest that heating may not significantly alter nutrient fractions (Rocha et al., 2014). Newkirk et al. (2003) noted that non-heat-treated canola meals might contain higher levels of digestible amino acids. It is possible that heating's effect on digestibility is minimal or that different heating processes are needed for optimal hempseed digestibility.

Digestibility of nutrients is affected by gut microflora and exogenous enzyme supplementation (Bedford and Cowison 2012). According to Lazaro et al (2004) enzyme supplementation mainly enhances performance by improving nutrient digestibility (Lazoroet al 2004). Evidently the enzyme supplementation (to raw and heated hemp) had improved all digestibility parameters. Yet this was not reflected in growth and feed efficiency as may arguable expected. It has to be noted that the digestibility trial was in last 4 days of experiment while growth performances criteria was over a 3 weeks period. It may a positive correlation was observed If the trial was conducted over the longer period. It may also be that the extent of digestibility was not suffice enough to be reflected in performance parameters.

Age plays a crucial role in digestibility issues (Wang et al 2021). Lu et al (2013) reported lower nutrient digestibility values for younger broiler chickens. Young birds have a less developed digestive tract, cannot produce enough enzymes on their own and may not tolerate high fiber diet (Olkusi et al (2007). According to Jozefiak et al. 2004) during the starter phase, undigested fiber limits the accessibility of digestive enzymes to feed substrates. Exogenous enzyme supplementation overcomes these short-comings, reduces the requirement for the enzyme and makes more nutrients and energy available for chicks growth. However, the beneficial effect of exogenous supplementation diminishes as chickens get older (Olukosi et al., 2007). Wang et al (2021) reported that chicks benefit more from enzyme addition at a younger age and that the contribution of enzymes to nutrient retention decreases with age in chickens.

CONCLUSIONS

Raw hempseed can be promising and beneficial in broiler feeding, improving performance and feed intake. However, heat-treated hempseed and adding enzymes did not offer additional benefits beyond those provided by raw hempseed alone. Exogenous enzyme supplementation did improve all digestibility parameters, while heat treatment of hempseed reduced protein digestibility. Further research is needed to evaluate the effects of higher hempseed inclusion levels and varying types and doses of enzyme supplementation at older ages on broiler chicken performance. Additionally, efforts could also focus on optimizing heating programs to reduce anti-nutritional factors and improve the nutritional digestibility of hempseed.

ETHICAL APPROVAL

The experimental protocols were approved (IR.MODARES.REC.1400.032) by the Biomedical Research Ethics Committee of Tarbiat Modares University.

Acknowledgements

We would like to thank AB Vista for supplying the enzymes used in this study.

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Table 1. Chemical composition of the raw hempseed used for the formulation of the diets (as-is basis).

Item	Content
Dry matter, %	94.62
Gross energy, kcal/kg	5925
Crude protein, %	24.7
Ether extract, %	30.5
Ash, %	5.37
Crude fiber, %	29.6
Neutral detergent fiber, %	32.4
Acid detergent fiber, %	22.1
Total Lysine, %	1.02
Total Methionine, %	0.43
Total Threonine, %	0.62

Table 2. Composition and nutrient contents of experimental diets (as-fed basis) offered d 8-28.

Ingredients (kg/ton feed)	Control diet	Raw hempseed diet	Heat-treated hempseed diet
Corn	558.65	536	536
Soybean meal (43%)	374	320	320
Hemp	0	100	100
Vegetable oil	27	0	0
Dicalcium phosphate	17.3	16	16
Limestone	9.8	10.5	10.5
Salt	1.8	1.75	1.75
NaHco3	2.75	2.85	2.85
DL-Methionine	2.55	2.8	2.8
L-Lysine HCl	0.9	1.45	1.45
L-Threonine	0.25	0.6	0.6
Vitamin/Mineral premix[a]	5	5	5
Filler	0	3.05	3.05
Calculated nutrient composition			
ME (kcal/kg)	2950	2950	2950
Crude protein (%)	20.52	20.59	20.59
Ca (%)	0.87	0.87	0.87
Available p (%)	0.44	0.44	0.44
Na (%)	0.16	0.16	0.16
Lysine (%)	1.18	1.18	1.18
Methionine+Cystine (%)	0.9	0.9	0.9
Threonine (%)	0.8	0.8	0.8
Analyzed nutrient composition[b]			
Moisture (%)	7.93	7.18	6.70
Crude protein (%)	20.4	20.18	20.26
Ash (%)	6.26	6.08	6.05
Ether extract	7.06	7.11	7.40

[a] Each kg of vitamin and mineral premix contained: Vitamin A 4000000 IU, vitamin E 26000 IU, vitamin D3 1800000 IU, vitamin K 1200 mg, vitamin B1 1000 mg, vitamin B2 2600 mg, Niacin 5400 mg, Pantothenic Acid 7500 mg, vitamin B6 1280 mg, Folic acid 760 mg, Biotin 72 mg, vitamin B12 6.8 mg, choline choloride 320000 mg and antioxidant 1000 mg, Fe, 8000 mg, Mn, 48000 mg, Cu, 6400 mg, I, 500 mg, Zn, 44000 mg, Se, 120 mg. [b] Analyzed according to the AOAC (1995).

Table 3. Effects of dietary treatments on growth performance (d 7-28).

Treatment[1]	Body weight, g	Body weight gain, g	Feed intake, g	Feed conversion ratio	Viability
Control	1003 [b]	852.1 [b]	1233 [b]	1.447	91.6
RH	1044 [a]	896.7 [a]	1278 [a]	1.425	95.0
RHE	1041 [a]	892.2 [a]	1280 [a]	1.435	96.6
HH	1027 [a]	879.5 [a]	1270 [a]	1.444	96.6
HHE	1023 [ab]	874.7 [a]	1259 [ab]	1.439	95.0
SEM	7.00	7.01	9.25	0.01	2.08
P values	0.002	0.001	0.009	NS[3]	NS
Process					
RH	1042 [a]	894.4 [a]	1279	1.44	95.8
HH	1025 [b]	877.2 [b]	1264	1.43	95.8
SEM	5.34	5.28	6.30	0.007	1.53
P-value	0.034	0.031	NS	NS	NS
Enzyme					
E0[2]	1035	888.1	1274	1.43	95.8
E1[2]	1032	883.5	1269	1.44	95.8
SEM	5.34	5.28	6.30	0.007	1.53
P-value	NS	NS	NS	NS	NS
Process×Enzyme					
SEM	7.55	7.47	8.19	0.01	2.17
P-value	NS	NS	NS	NS	NS

[1] Control (no hempseed), RH= 10% raw hempseed in the diet, RHE= 10% RH with enzyme supplementation, HH= 10% heat-treated hempseed in the diet, HHE= 10% HH with enzyme supplementation
[2] E0= Without Enzyme; E1= With Enzyme.
[3] NS= Not Significant.

Table 4. Effects of dietary treatments on microflora composition of ileum (log 10 CFU/g) at day 28 of broilers.

Treatment[1]	E. coli	Total Aerobes	Lactobacillus spp.
Control	10.06 ^{a}	9.49 ^{a}	8.62 ^{c}
RH	9.23 ^{b}	9.68 ^{a}	8.87 ^{b}
RHE	8.75 ^{c}	9.17 ^{b}	8.97 ^{b}
HH	9.41 ^{b}	8.93 ^{b}	9.32 ^{a}
HHE	8.77 ^{c}	9.57 ^{a}	9.37 ^{a}
SEM	0.086	0.099	0.070
P-value	<0.001	<0.001	<0.001
Process			
RH	8.99	9.42	8.92 ^{b}
HH	9.09	9.25	9.35 ^{a}
SEM	0.060	0.072	0.052
P-value	NS	NS[3]	<0.001
Enzyme			
E0[2]	9.32 ^{a}	9.31	9.10
E1[2]	8.76 ^{b}	9.37	9.17
SEM	0.060	0.072	0.052
P-value	<0.001	NS	NS
Process×Enzyme			
SEM	0.085	0.102	0.074
P-value	NS	<0.001	NS

[1] Control (no hempseed), RH= 10% Raw Hempseed in the diet, RHE= 10% RH with enzyme supplementation, HH= 10% Heat-treated Hempseed in the diet, HHE= 10% HH with enzyme supplementation.
[2] E0= Without Enzyme; E1= With Enzyme.
[3] NS= Not Significant.

Table 5. Effects of dietary treatments on ileal digestibility.

Treatment [1]	DM	Organic matter, %	Fat, %	NDF[2], %	ADF[2], %	Crude protein	Gross energy
Control	60.78 ^{b}	61.41 ^{b}	69.91 ^{b}	27.31 ^{c}	15.24 ^{bc}	67.66 ^{c}	62.59 ^{b}
RH	60.80 ^{b}	61.30 ^{b}	71.59 ^{b}	29.39 ^{bc}	14.28 ^{c}	65.62 ^{c}	60.50 ^{b}
RHE	62.85 ^{a}	69.25 ^{a}	79.64 ^{a}	34.05 ^{a}	19.32 ^{a}	78.26 ^{a}	70.16 ^{a}
HH	60.75 ^{b}	63.27 ^{b}	72.99 ^{b}	27.90 ^{c}	13.92 ^{c}	64.97 ^{c}	61.32 ^{b}
HHE	63.16 ^{a}	70.33 ^{a}	78.64 ^{a}	31.06 ^{b}	17.86 ^{b}	72.61 ^{b}	71.20 ^{a}
SEM	0.284	0.921	0.945	0.947	1.070	1.301	1.506
P valus	<0.001	<0.001	<0.001	0.003	0.019	<0.001	<0.001
Process							
RH	0.946	65.27	75.61	31.71	16.80	71.94 ^{a}	65.33
HH	61.95	66.79	75.81	29.48	15.89	68.79 ^{b}	66.26
SEM	0.225	0.728	0.734	0.742	0.788	0.967	1.19
P-value	NS[4]	NS	NS	NS	NS	0.049	NS
Enzyme							
E0[3]	60.77 ^{b}	62.28 ^{b}	72.28 ^{b}	28.64 ^{b}	14.10 ^{b}	65.29 ^{b}	60.91 ^{b}
E1[3]	63.00 ^{a}	69.79 ^{a}	79.13 ^{a}	32.55 ^{a}	18.59 ^{a}	75.43 ^{a}	70.68 ^{a}
SEM	0.225	0.728	0.734	0.742	0.788	0.967	1.19
P-value	0.001	<0.001	0.002	0.005	0.003	<0.001	0.004
Process * Enzyme							
SEM	0.318	1.03	1.04	1.05	1.11	1.36	1.68
P-value	NS	NS	NS	NS	NS	NS	NS

[1] Control (no hempseed), RH= 10% raw hempseed in the diet, RHE= 10% RH with enzyme supplementation, HH= 10% heat-treated hempseed in the diet, HHE= 10% HH with enzyme supplementation.
[2] ADF= Acid Detergent Fiber; NDF= Neutral Detergent Fiber.
[3] E0= Without Enzyme; E1= With Enzyme.
[4] NS= Not Significant.