

AN ABSTRACT OF THE THESIS OF

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Title: Canola meal as a protein supplement in dairy  
rations

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A four months continuous feeding trial was conducted to evaluate Canola meal as a single protein supplement in complete dairy rations. Thirty high producing Holstein cows in the second week of lactation were randomly allotted in groups of ten to one of three isonitrogenous (15% CP) and isocaloric (1.6 Mcal/kg of NE lactation) rations, containing either Canola meal (38% CP), Soybean meal (46% CP) or Cottonseed meal (41% CP). Productive and physiological parameters were analysed.

Milk production, both actual and 4% FCM, did not differ ( $P > .05$ ) among diets. However, Canola meal fed cows tended to produce more milk than animals in other treatments. No differences ( $P > .05$ ) were found for milk protein, fat, total solids nor solids not fat percentages. Milk solids not fat yield was the only milk component produced in different ( $P = .024$ ) amounts between diets and was mainly influenced by milk production.

Canola meal glucosinolates, tannins and phytate did not impair feed intake, however animals in this group

showed lower feed conversion.

Rumen total volatile fatty acid content was higher ( $P=.031$ ) for Canola meal and Soybean meal groups. No significant differences ( $P>.05$ ) were found for rumen volatile fatty acid composition, acetic:propionic ratio, ammonia nitrogen or pH.

Urea nitrogen, total protein and albumin plasma concentrations were not different ( $P>.05$ ) among diets, suggesting that Canola, Soybean and Cottonseed meals were equal as protein supplements.

White blood cells was the only hematological parameter (packed cell volume, red blood cells, white blood cells and hemoglobin were analysed) that significantly differed ( $P=.011$ ) among diets. Soybean meal fed cows showed higher white blood cell count, which is believed to have a physiological origin.

Triiodothyronine uptake values were significantly higher ( $P=.015$ ) in Soybean meal fed cows. However, Triiodothyronine uptake as well as Tetraiodothyronine concentration and Free Thyroxine Index correspond to euthyroid values, indicating that Canola meal glucosinolates did not affect the thyroid metabolism of the experimental animals.

Results indicate that Canola meal may be used as a single protein supplement in rations for high producing cows, to satisfactorily replace traditional sources of protein such as Soybean meal and Cottonseed meal.

Canola meal as a protein supplement in dairy rations

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## TABLE OF CONTENTS

Abstract	1
Introduction	3
Experimental procedure	7
Results and discussion	12
Conclusion	24
Bibliography	25
Appendices	28

## LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Composition of the complete rations	17
2	Least square means for lactation performance and body weight changes for cows fed rations containing Soybean, Cottonseed or Canola meals as supplemental protein	18
3	Average body weight, feed intake and feed conversion for cows fed with different protein supplements	19
4	Influence of different protein sources on ruminal volatile fatty acids, ammonia nitrogen and pH	20
5	Effect of different protein sources on blood chemistry variables	21
6	Least square means for hematological variables for cows fed with different protein sources	22
7	Least square means for Thyroid hormones for cows fed with Soybean, Cottonseed or Canola meals as protein sources	23

## CANOLA MEAL AS A PROTEIN SUPPLEMENT IN DAIRY RATIONS

### ABSTRACT

Thirty high producing Holstein cows in the second week of lactation were used in a four months feeding trial to compare Canola meal with Soybean and Cottonseed meals as single protein supplements in complete dairy rations. Diets were isonitrogenous and isocaloric and contained Canola, Cottonseed or Soybean meals in levels of 11.7, 10.4 or 8.6%, respectively.

Actual milk production and 4% FCM did not differ ( $P>.05$ ) among diets. However, Canola meal fed cows tended to yield more milk than cows in Soybean meal or Cottonseed meal groups. Milk components (protein, fat, total solids and solids not fat) percentages were not different ( $P>.05$ ) among treatments. Milk solids not fat was the only milk component produced in different amounts ( $P=.024$ ) reflecting the accumulated differences in milk production and percentage solid not fat. Different diets did not affect ( $P>.05$ ) milk flavor quality.

No differences ( $P>.05$ ) were found for body weight changes. Average group feed intake was similar for cows on different diets.

Rumen total Volatile Fatty Acid content was higher ( $P=.031$ ) for Canola and Soybean meal groups, other rumen fluid parameters did not differ ( $P>.05$ ) among experimental groups.



Plasma urea nitrogen, total protein and albumin, as well as packed cell volume, red blood cell count and hemoglobin content did not appear to be affected by rations. Leucocytic values were higher for soybean meal fed cows but these were not considered to be affected by the diet.

Thyroid function analysis (Triiodothyronine uptake, Tetraiodothyronine and Free Thyroxine Index) showed normal values and indicate no effect of Canola glucosinolates on Thyroid gland.

Analysis of productive and physiological parameters suggest that Canola meal may be used in levels in the order of 12% in complete rations to feed cows capable of producing an average of 32kg of 4% FCM per day during the first half of lactation.

## INTRODUCTION

Joint efforts of plant breeders and animal nutritionists have widely expanded the usage of Canadian Rape-seed Meal as a supplemental source of protein for livestock, through the development of low glucosinolate cultivars. Tower, Regent, Candle and Altex are low glucosinolate cultivars presently used. Meals obtained after the extraction of the oil from the seed of these new varieties receive the common name of Canola Meal (CM) to distinguish them from traditional Rapeseed Meal (RSM) high in glucosinolates and processed by methods that render a meal of inferior feed quality. CM is characterized as containing less than 3 mg of glucosinolates (goitrogenic compound) per gram (6).

Protein percentages of CM depends on the cultivar from which the meal is produced. Candle cultivars render a product of 35% Crude Protein (CP) and Tower, Regent and Altex meals have 38 to 39% CP. Amino acid composition of the meal is comparatively lower in Lysine and higher in Sulphur containing amino acids than Soybean meal (SBM). Ether extract content is in the order of 4%. This value is higher in CM than in other oilmeals especially because of the inclusion of 1.5% of the gums obtained during the refinement of Canola oil. Available Ca, Fe, Mn, P, Se, and Mg is higher in CM than in SBM; however, high content of phytic acid and crude fiber in

the meal limit Cu, Zn and K availability.

Most research conducted using low glucosinolate Rapeseed Meal (LGRSM) in levels up to 26% in the grain supplement have shown that this protein source can sustain milk production at the same level as traditional protein supplements. Glucosinolate content in these rations did not impair feed intake (11, 15, 21, 24, 27). Laarveld and Christensen (15) have observed that cows fed grain supplements containing 17% 1788 RSM (low glucosinolate cultivar) tend to produce more milk than control animals. However, Fisher and Walsh (8) have reported that milk production declined when cows were fed more than 11% LGRSM in the grain supplement. Analysis of milk composition indicates that LGRSM does not significantly affect fat, protein, total solids nor milk solids not fat (SNF) (11, 14). Milk composition alterations (protein and fat production) obtained in previous investigations (8) could be due to the use of low quality meals produced under experimental processing techniques. These experiments have been carried out during 28 day periods of time and the ability of the lactating cow to adapt to short periods of stress by removing body deposits could mask any nutritional unbalance. Therefore, Canola meal should be analysed in longer term experiments, evaluating different phases of the lactation curve, feed utilization and health problems.

Red and white blood cell counts are the hematological parameters that have significantly differed among diets containing LGRSM and SBM. Laarveld and Christensen (15) and Papas, et al (21) have reported reductions and increases in white blood cell count, respectively. Discrepancy between both values may be due to blood samplings done during different phases of the physiological response of the animals to stress conditions. Papas, et al (21) have observed a decline in red blood cell count in animals fed LGRSM diets.

Analysis of rumen fluid conducted by Laarveld and Christensen (15), Ingalls and Sharma (11) and Sharma, et al (24) showed that the inclusion of LGRSM in levels up to 25% in the grain supplement does not affect pH, ammonia content nor volatile fatty acid (VFA) composition.

No conclusive results have been published about the goitrogenic potential of glucosinolate content in CM on lactating cows. Fisher and Walsh (8) have reported a significant reduction in Thyroxine (T4) serum concentration in cows fed a grain supplement containing 34% 1788 RSM for 28 days; however Triiodothyronine (T3) serum concentration and T4 to T3 conversion did not significantly differ from the control diet (RSM). In a similar experiment Sharma, et al (24) feeding grain supplements containing 25% commercial RSM (high glucosinolate meal) and 25% 1788 RSM obtained significantly lower values of T4

serum concentration only in cows fed 1788 RSM. These results may be considered ambiguous because of the failure of the high glucosinolate RSM to alter the thyroid metabolism as did 1788 RSM. In these studies goitrogenic effect of glucosinolates was analysed using T3 and T4 serum concentrations and the results suggest the insensitivity of the analysis and the need of using more suitable techniques for studying the thyroid metabolism. Laarveld, et al (14) in a recent experiment, used the Thyrotropin-releasing Hormone (TRH) test. Results showed that Tower at levels of 18.9% of the total diet did not affect the response of plasma Thyroid Stimulating Hormone (TSH), T4 or T3 concentrations to TRH injection. However, TRH test caused a significant increase in TSH and a significant reduction in T4 serum concentration, in cows fed diets containing more than 13% of Midas RSM (high glucosinolate meal). Levels of T3 and T3:T4 ratios did not differ between Tower, Midas and control (SBM) groups.

The present research was conducted to further analyse the utilization of Canola Meal as a single protein supplement in commercial dairy rations fed to high producing cows in early lactation by evaluating productive and physiological parameters.

## EXPERIMENTAL PROCEDURE

Thirty high producing Holstein cows in the second or later lactation were used in a four months continuous feeding trial of a randomized block design (7). Selected animals were arranged according to expected date of calving and groups of 6 cows due with an 8 day period were randomly allotted in sets of 2 to each of the 3 experimental diets. Groups of cows in the second week of lactation were simultaneously placed in each treatment group during a 10 week period.

Complete rations were isocaloric (1.6 Mcal/kg of NE of lactation) and isonitrogenous (15% CP) and contained as a single protein supplement either SBM (46% CP), Cottonseed Meal (CSM) (41% CP) or CM (38% CP) (20). CM used was a commercial blend of Candle and Tower varieties, containing 38% CP. Diets differed mainly in the protein supplement and their composition is shown in Table 1. Cows were fed in amounts that exceeded the previous day's intake once a day between 0730 and 0830 h. Twice a week, rejected feed was weighed back and feed intake was calculated for each group of cows. Samples of feed were analysed biweekly for dry matter, CP, Ca and P, according to AOAC procedures (2) and Acid Detergent Fiber (ADF) was determined by the method of Van Soest (28), (Table 1). Feed conversions were estimated by establishing the relationship between milk production and feed intake, and milk

production and protein intake. Animals were weighed upon entering the experiment and every month thereafter to determine changes in body weight.

Animals were housed under cover in a free stall loafing barn. Cows were milked twice a day and milk yields measured. Milk samples from two consecutive milkings were pooled and the composite sample was tested biweekly for milk flavor quality, protein, fat and total solids. Milk flavor quality was analysed in samples tempered at 10°C by a 2 person panel of experienced judges using the American Dairy Science Association Official Score Card for fluid milk (18), which ranges from 1 (unpalatable) to 10 (no criticism). Milk protein percentage was determined by a colorimetric Orange G dye binding method modified by Ashworth, et al (1), milk fat percentage was determined by the BANCO<sup>1</sup> detergent procedure, and total solids percentage by the Semi-Automatic Rapid Moisture Test (19), drying the samples in a Brabender forced air system oven at 130°C during a period of 40 minutes. From these data were calculated SNF percentages and absolute values for milk production corrected to 4% fat (4%FCM), total solids, solids not fat, fat and protein production per day.

Cows in the fourth month of research were sampled

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<sup>1</sup>Anderson Laboratories, Inc. Fort Worth, TX 76112.

for blood and rumen fluid 6 hours after morning feeding. Blood samples were drawn from the coccygeal vessel into heparinized tubes by tail puncture, and placed on ice until analysed for hemoglobin by a cyanmethemoglobin method using a Coulter hemoglobinometer. Red and white blood cell counts were determined with a Coulter electronic cell counter (model ZBI), and packed cell volume by the microhematocrit method. A second portion of the blood sample was centrifuged at 755g for 20 minutes and the plasma separated and stored at -20°C for subsequent determination of T4, employing a Quantitope<sup>125</sup>I Thyroxine Radioimmunoassay Kit<sup>2</sup>, which uses a double antibody complex as a binder and <sup>125</sup>I labeled Thyroxine as the radioactive tracer. Triiodothyronine uptake (RT3%) was analysed using a TRI-TAB T3 Uptake Diagnostic Kit<sup>3</sup> to determine the binding capacity of unsaturated Thyroxine-binding globulin. Free Thyroxine Index was calculated by multiplying the T4 concentration by the Triiodothyronine uptake percentage. The same samples of plasma were tested for Urea Nitrogen (PUN) by the colorimetric measure of the product of the reaction of Diacetyl Monoxime and Thiosemicarbazide with urea under acidic conditions (Pierce Urea Nitrogen Rapid

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<sup>2</sup>Kallestad Laboratories Inc. 2000 Austin National Bank Tower, Austin, TX 78701.

<sup>3</sup>Nuclear-Medical Laboratories Inc. Dallas, TX 75247.

<sup>4</sup>Pierce Company. Box 117, Rockford, IL 61105.



Stat Kit<sup>4</sup>). Analysis of albumin and total protein was done by the colorimetric determination of orange and lavender complexes resulting from the reactions of albumin with a yellow dyestuff and total protein with a blue cupric tartrate complex, respectively (Pierce Total protein/Albumin Rapid Stat Kit<sup>4</sup>).

Rumen fluid samples were obtained from the cows by applying vacuum to an esophageal tube. Samples were strained through cheesecloth and fresh rumen fluid was analysed for pH in an Orion Research model 701 A meter, and for ammonia content using a modified aeration method by Van Slyke and Cullen (10). For further analysis of VFA 5 ml of rumen fluid were treated with 1 ml of 25% metaphosphoric acid, the solution was let stand for 30 minutes and then clarified by centrifugation at 18800 g for 20 minutes. Supernatant was decanted and stored at -20°C. Determination of VFA concentration was conducted on a Varian Aerograph Series 1200 Gas Chromatograph connected to a Spectra-Physics Minigrator for the integration of the peak area corresponding to the VFA analysed. The chromatographic column was packed with Chromosorb 101<sup>5</sup> of 80/100 mesh size and the standard used was a rumen VFA solution prepared by Supelco Inc<sup>6</sup>. Concentration of total and individual VFA were measured and the

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<sup>5</sup>Johns-Manville. Denver, CO 80217.

<sup>6</sup>Supelco Inc. Supelco Park. Bellefonte, PA 16823.

relative composition and Acetic:Propionic acids ratio was calculated.

Data collected for different variables were analysed using Least Squares Analysis of Variance for unbalanced number of observations (9). Means were compared using the LSD method as described by Steel and Torrie (25).

## RESULTS AND DISCUSSION

Milk yield and composition are summarized in Table 2. Milk production, both actual and 4% FCM, were not different ( $P>.05$ ) among treatments. However, linear contrast comparison showed that actual milk production was 3.22 and 1.17 kg/day, and 4% FCM was 4.06 and 2.42 kg/day higher in CM fed cows than in SBM or CSM fed animals, respectively. Similar trends to higher milk production in cows fed CM have been reported by Fisher and Ingalls (unpublished data), Laarveld and Christensen (15), and Papas, et al (21). Protein, fat, total solids and milk SNF percentages did not differ ( $P>.05$ ) among diets. Milk SNF yield was the only milk component that differed between treatments. Cows fed CM produced 0.57 and 0.28 kg/day of SNF more than animals in SBM or CSM groups, respectively. These differences reflect the cumulative effect of higher milk production and higher SNF percentages in milk of cows fed CM (Table 1). Sharma, et al (24) have also reported that CM does not affect ( $P>.05$ ) milk composition, and differences in milk component production among diets have the same trend as milk production.

There was no treatment effect on milk flavor quality (Table 2). This demonstrates that Canola oil residues and gums added to the meal did not affect ( $P>.05$ ) organoleptic properties of milk.

There were no differences ( $P>.05$ ) in body weight

changes between groups. Cows in all treatments tended to gain weight during the experimental period.

Mean feed intake was higher for CM fed animals than for other groups (Table 3). Previous research of Fisher and Ingalls (unpublished data), and others (21, 24) have also demonstrated that glucosinolates, tannins or phytate content in complete rations containing up to 14% of CM do not impair feed intake. Feed conversions expressed as milk production:feed intake and milk production:protein intake showed that SBM and CSM fed cows had more efficient feed utilization.

Rumen total VFA content (Table 4) was higher ( $P=.031$ ) in CM and SBM groups than in the CSM group. According to Church (4) these values are relatively low and they may indicate saliva contamination during rumen fluid sampling by the stomach tube technique. Molar percentage of VFA was not changed for different diets. Total rumen VFA and its composition agree with reported values (15, 24).

Higher ADF content in CM rations did not appear to affect ( $P>.05$ ) the acetic:propionic ratio (Table 4) nor milk fat yield (Table 2). Results show that new varieties of LGRSM lower in ADF and higher in CP are better alternative protein supplements than varieties with high ADF content.

Rumen fluid pH (Table 4) values are in the range for optimal proteolytic activity of rumen bacterial (4) and

were not modified ( $P > .05$ ) by diets.

Rumen ammonia nitrogen content (Table 4) coincides with results reported by Ingalls and Sharma (11) and Sharma, et al (24) and suggest that CM nitrogen is released in the rumen at a similar rate as SBM.

PUN, total serum protein and albumin values are shown in Table 5. No effect ( $P > .05$ ) of the rations on these parameters was found. Values are in the normal range for lactating cows (5). Higher plasma total protein and plasma albumin in cows fed Cottonseed meal suggest that this oilmeal supplied more nitrogen to the protein metabolism of the experimental animals (13). Laarveld and Christensen (15) and Sharma, et al (24) have found analogous values for these parameters in cows fed CM or SBM.

Swenson (26) has reported that the nutritional status of the animal affects PCV, RBC and hemoglobin content. No differences ( $P > .05$ ) were found for these variables (Table 6) and values are normal (23). Results agree with Iwarsson (12) who did not find effects of glucosinolates on these hematological parameters.

WBC counts were higher ( $P = .011$ ) for SBM than for CM or CSM fed cows. Fifty percent of the SBM-fed cows showed leucocytosis, which is believed to have a physiological origin (stress reaction), (Table 6) since animals were in good health throughout the experimental period.

Thyroid hormone analysis is shown in Table 7.

Antithyroid and goitrogenic activity of RSM has been attributed to hydrolytic products of glucosinolates (oxazolidine-thione-OZT- and isothiocyanate-BNCS-)(3).

Lo and Bell (16) have proved that OZT causes goiter and BNCS reduces T3 and T4 content in the thyroid gland. Joint effect of both toxicants produces higher proportions of iodine remaining as iodide, as well as decreasing proportions of T3+T4: monoiodothyrosine-diiodothyrosine. According to Miller, et al (17) this reduction in the incorporation of iodine to organic compounds in animals fed feedstuffs containing thiocyanates is responsible for decreasing secretion of iodine in milk. All the animals showed T3 uptake levels within the euthyroid range (35-45%)(Table 7) of Tri Tab T3 uptake method. Lower ( $P < .05$ ) T3 uptake levels for cows fed CM could be due to the effect of glucosinolates on the thyroid gland. However, different ( $P < .05$ ) values among diets containing no glucosinolates (SBM and CSM) and the similarity between CM and CSM groups raises question about the accuracy of the Tri-Tab T3 uptake method to analyse thyroid activity in cows. There was no treatment effect on plasma T4 levels nor FTI (Table 7).

T4 values are similar to results obtained by Papas, et al (21) and Laarveld and Christensen (15) using Tower and 1821 RSM in dairy rations. FTI was mainly determined by T4, values are in the euthyroid range for humans

(0.9-2.9) (22). Rosefeld (22) has demonstrated the high correlation ( $r = .93$ ) between FTI and the more elaborated "free" Thyroxine assay, use to measure the free form of thyroxine that diffuses into the cell to regulate cellular metabolism. Throid hormone analysis suggests that use of CM in 11.7% of rations does not affect the thyroid activity when fed to dairy cows during a four months period.

TABLE 1. COMPOSITION OF THE COMPLETE RATIONS.

COMPONENT	Soybean Meal (SBM)	Cottonseed Meal (CSM)	Canola Meal (CM)
	% as fed		
Corn Silage	30.0	30.0	30.0
Alfalfa Hay	20.0	20.0	20.0
SBM	8.6	--	--
CSM	--	10.4	--
CM	--	--	11.7
Corn	9.7	13.2	17.0
Oats	30.2	25.0	20.0
Salt	0.25	0.25	0.25
Trace Minerals and Vitamins	0.50	0.50	0.50
Dicalcium Phosphate	0.30	--	0.10
Limestone	0.50	0.70	0.45
	% of Dry Matter		
Protein	15.1	15.1	15.2
ADF	22.6	22.9	24.0
Calcium	0.59	0.59	0.66
Phosphorus	0.35	0.39	0.40
NE Lactation (Mcal/kg) *	1.6	1.6	1.6

\* Estimated Value.



TABLE 2. LEAST SQUARE MEANS FOR LACTATION PERFORMANCE AND BODY WEIGHT CHANGE FOR COWS FED RATIONS CONTAINING SOYBEAN, COTTONSEED OR CANOLA MEALS AS SUPPLEMENTAL PROTEIN.

Variable	N <sup>o</sup> of animals	Soybean Meal	Cottonseed Meal	Canola Meal	SEM	P
Milk kg/day	10	34.45	36.50	37.67	0.979	0.120
FCM, kg/day	10	28.07	29.71	32.13	0.730	0.103
Milk protein, kg/day	10	1.01	1.07	1.15	0.0291	0.180
Milk protein, %	10	2.95	3.02	2.96	0.0374	0.702
Milk fat, kg/day	10	0.95	1.02	1.08	0.0262	0.129
Milk fat, %	10	2.70	2.76	2.63	0.0429	0.505
Milk total solids, kg/day	10	4.06	4.35	4.71	0.122	0.122
Milk total solids, %	10	12.02	12.06	12.01	0.181	0.993
Milk solids not fat, kg/day	10	3.13 <sup>a</sup>	3.42 <sup>b</sup>	3.70 <sup>c</sup>	0.0749	0.024
Milk solids not fat, %	10	9.21	9.24	9.30	0.165	0.974
Milk flavor quality	10	7.96	8.09	7.74	0.0861	0.309
Body weight change, kg/day	10	0.49	0.36	0.38	0.0539	0.582

<sup>a,b,c</sup> Means in the same row with different superscripts are different (P<.05)

TABLE 3. AVERAGE BODY WEIGHT, FEED INTAKE AND FEED CONVERSION FOR COWS  
FED WITH DIFFERENT PROTEIN SUPPLEMENTS.

Variable	Soybean Meal	Cottonseed Meal	Canola Meal
Body weight, kg	666	651	693
Dry matter intake, kg	18.1	19.6	21.2
Feed conversion			
Feed intake:milk production	1.98	1.88	1.84
Milk production:protein intake	13.09	12.41	12.08

TABLE 4. INFLUENCE OF DIFFERENT PROTEIN SOURCES ON RUMINAL VOLATILE FATTY ACIDS, AMMONIA NITROGEN AND pH.

Variable	N <sup>o</sup> of animals	Soybean Meal	Cottonseed Meal	Canola Meal	SEM	P
Total Volatile Fatty Acids m moles/dl	8	10.2 <sup>b</sup>	8.9 <sup>a</sup>	10.7 <sup>b</sup>	0.218	0.031
Acetic acid, %	8	64.1	63.1	64.1	1.25	0.935
Propionic acid, %	8	17.1	21.1	19.6	1.44	0.575
Isobutyric acid, %	8	1.3	1.0	1.6	0.132	0.239
N-Butyric acid, %	8	14.2	12.0	12.5	0.142	0.065
Isovaleric acid, %	8	1.4	1.2	1.1	0.139	0.647
N-Valeric acid, %	8	1.4	1.6	1.4	0.0564	0.481
Acetic:Propionic ratio	8	4.2	3.0	3.6	0.369	0.506
Ammonia Nitrogen, mg/dl	8	7.9	9.1	8.1	0.360	0.327
pH	8	6.45	6.61	6.23	0.140	0.404

<sup>a,b</sup> Means in the same row with different superscripts are different (P<.05)

TABLE 5. EFFECT OF DIFFERENT PROTEIN SOURCES ON BLOOD CHEMISTRY VARIABLES.

Variable	N <sup>o</sup> of animals	Soybean Meal	Cottonseed Meal	Canola Meal	SEM	P
Plasma urea nitrogen, mg/dl	9	16.23	16.29	17.44	0.980	0.852
Plasma total protein, g/dl	9	11.92	12.48	10.47	0.477	0.296
Plasma albumin, g/dl	9	3.13	3.47	3.06	0.0992	0.289

TABLE 6. LEAST SQUARE MEANS FOR HEMATOLOGICAL VARIABLES FOR COWS FED WITH DIFFERENT PROTEIN SOURCES.

Variable	N <sup>o</sup> of animals	Soybean Meal	Cottonseed Meal	Canola Meal	SEM	P
Packed Cell Volume, %	6	31.1	29.1	29.8	0.607	0.312
Red blood cells, $\times 10^6/\text{mm}^3$	6	6.22	6.69	5.67	0.376	0.447
White blood cells, $\times 10^3/\text{mm}^3$	6	28.57 <sup>a</sup>	10.65 <sup>b</sup>	11.72 <sup>b</sup>	1.90	0.011
Hemoglobin, g/dl		11.26	11.54	11.77	0.307	0.721

<sup>a,b</sup> Means in the same row with different superscripts are different ( $P < .01$ )

TABLE 7. LEAST SQUARE MEANS FOR THYROID HORMONES FOR COWS FED WITH SOYBEAN, COTTON-SEED OR CANOLA MEALS AS PROTEIN SOURCES.

Sources	N <sup>o</sup> -of animals	Soybean Meal	Cottonseed Meal	Canola Meal	SEM	P
Triiodothyronine uptake, %	4	43.31 <sup>a</sup>	41.29 <sup>b</sup>	41.94 <sup>b</sup>	0.155	0.015
Thyroxine, mcg/dl	4	4.64	4.20	5.35	0.334	0.386
Free thyroxine index	4	2.01	1.74	2.24	0.139	0.354

<sup>a,b</sup> Means in the same row with different superscripts are different (P<.05)

## CONCLUSION

Evaluated productive and physiological parameters showed that Canola, Cottonseed and Soybean meals were equal as protein supplements in rations for high producing dairy cows. Therefore, Canola meal may be used as a single protein supplement in levels in the order of 12% of the complete ration.

Further research is recommended to evaluate the usage of Canola meal in rations with different protein percentages, and to better understand feed utilization in cows fed this feedstuff, considering the metabolizable protein feeding standard.

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## APPENDICES

TABLE 1. ANALYSIS OF VARIANCE FOR ACTUAL MILK PRODUCTION PER DAY.

Source	D.F.	M.S.	F.	P.
Treatments	2	245.215	2.54	0.120
Blocks	3	364.924	3.78*	0.041
Age groups	2	37.406	0.39	
Treatments X blocks	6	200.900	2.08	
Treatments X age groups	4	40.421	0.42	
Remainder	12	96.545		
Total	29			

\* Significant ( $P < 0.05$ )

TABLE 2. ANALYSIS OF VARIANCE FOR FAT CORRECTED MILK PRODUCTION PER DAY.

Source	D.F.	M.S.	F.	P.
Treatments	2	148.511	2.77	0.103
Blocks	3	215.654	4.02*	0.034
Age groups	2	68.055	1.27	
Treatments X blocks	6	72.122	1.34	
Treatments X age groups	4	51.015	0.95	
Remainder	12	53.671		
Total	29			

\* Significant ( $P < .05$ )

TABLE 3. ANALYSIS OF VARIANCE FOR MILK PROTEIN PRODUCTION PER DAY.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.169	1.99	0.180
Blocks	3	0.218	2.57	
Age groups	2	0.045	0.53	
Treatments X blocks	6	0.199	2.34	
Treatments X age groups	4	0.064	0.75	
Remainder	12	0.085		
Total	29			

TABLE 4. ANALYSIS OF VARIANCE FOR MILK PRODUCTION PERCENTAGE.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.011	0.37	0.702
Blocks	3	0.050	1.72	
Age groups	2	0.001	0.04	
Treatments X blocks	6	0.044	1.50	
Treatments X age groups	4	0.072	2.47	
Remainder	12	0.029		
Total	29			

TABLE 5. ANALYSIS OF VARIANCE FOR MILK FAT PRODUCTION PER DAY.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.169	2.44	0.129
Blocks	3	0.280	4.05*	0.034
Age groups	2	0.144	2.08	
Treatments X blocks	6	0.123	1.78	
Treatments X age groups	4	0.091	1.32	
Remainder	12	0.069		
Total	29			

\* Significant ( $P < .05$ )

TABLE 6. ANALYSIS OF VARIANCE FOR MILK FAT PERCENTAGE.

Sources	D.F.	M.S.	F.	P.
Treatments	2	0.723	0.72	0.505
Blocks	3	0.155	4.07*	0.033
Age groups	2	0.034	0.90	
Treatments X blocks	6	0.378	9.94**	0.001
Treatments X age groups	4	0.035	0.92	
Remainder	12	0.038		
Total	29			

\* Significant ( $P < .05$ )

\*\* Significant ( $P < .01$ )

TABLE 7. ANALYSIS OF VARIANCE FOR MILK TOTAL SOLIDS PRODUCTION PER DAY.

Source	D.F.	M.S.	F.	P.
Treatments	2	3.792	2.53	0.122
Blocks	3	7.069	4.71*	0.021
Age groups	2	1.477	0.98	
Treatments X blocks	6	2.074	1.38	
Treatments X age groups	4	2.158	1.44	
Remainder	12	1.502		
Total	29			

\* Significant ( $P < .05$ )

TABLE 8. ANALYSIS OF VARIANCE FOR MILK TOTAL SOLIDS PERCENTAGE.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.005	0.01	0.993
Blocks	3	0.013	0.02	
Age groups	2	0.023	0.03	
Treatments X blocks	6	0.898	1.31	
Treatments X age groups	4	0.334	0.49	
Remainder	12	0.684		
Total	29			

TABLE 9. ANALYSIS OF VARIANCE FOR MILK SOLIDS NOT FAT PRODUCTION PER DAY.

Source	D.F.	M.S.	F.	P.
Treatments	2	2.925	5.18*	0.024
Blocks	3	3.561	6.31**	0.008
Age groups	2	0.232	0.41	
Treatments X blocks	6	2.474	4.38*	0.014
Treatments X age groups	4	0.470	0.83	
Remainder	12	0.565		
Total	29			

\* Significant ( $P < .05$ )\*\* Significant ( $P < .01$ )

TABLE 10. ANALYSIS OF VARIANCE FOR MILK SOLIDS NOT FAT PERCENTAGE.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.015	0.03	0.024
Blocks	3	0.187	0.33	
Age groups	2	0.108	0.19	
Treatments X blocks	6	0.275	0.49	
Treatments X age groups	4	0.071	0.13	
Remainder	12	0.565		
Total	29			



TABLE 11. ANALYSIS OF VARIANCE FOR MILK QUALITY.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.199	1.30	0.309
Blocks	3	0.213	1.39	
Age groups	2	0.173	1.13	
Treatments X blocks	6	0.037	0.24	
Treatments X age groups	4	0.057	0.37	
Remainder	12	0.154		
Total	29			

TABLE 12. ANALYSIS OF VARIANCE FOR BODY WEIGHT CHANGE.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.166	0.57	0.582
Blocks	3	0.898	3.07	
Age groups	2	1.060	3.62	
Treatments X blocks	6	0.551	1.88	
Treatments X age groups	4	0.601	2.05	
Remainder	12	0.292		
Total	29			

TABLE 13. ANALYSIS OF VARIANCE FOR RUMEN TOTAL VOLATILE FATTY ACIDS.

Source	D.F.	M.S.	F.	P.
Treatments	2	2.624	5.98*	0.031
Blocks	3	1.623	3.70	
Age groups	2	0.553	1.26	
Treatments X blocks	6	0.493	1.12	
Treatments X age groups	4	0.962	2.19	
Remainder	7	0.439		
Total	24			

\* Significant ( $P < .05$ )

TABLE 14. ANALYSIS OF VARIANCE FOR RUMEN ACETIC ACID.

Source	D.F.	M.S.	F.	P.
Treatments	2	1.402	0.07	0.935
Blocks	3	10.869	0.53	
Age groups	2	43.750	2.11	
Treatments X blocks	6	12.261	0.59	
Treatments X age groups	4	1.446	0.07	
Remainder	7	20.704		
Total	24			

TABLE 15. ANALYSIS OF VARIANCE FOR RUMEN PROPIONIC ACID.

Source	D.F.	M.S.	F.	P.
Treatments	2	16.521	0.60	0.575
Blocks	3	37.231	1.35	
Age groups	2	19.734	0.72	
Treatments X blocks	6	3.598	0.13	
Treatments X age groups	4	2.567	0.09	
Remainder	7	27.536		
Total	24			

TABLE 16. ANALYSIS OF VARIANCE FOR RUMEN ISOBUTYRIC ACID.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.407	1.77	0.239
Blocks	3	0.140	0.61	
Age groups	2	1.110	4.82*	0.048
Treatments X blocks	6	0.217	0.94	
Treatments X age groups	4	0.402	1.75	
Remainder	7	0.230		
Total	24			

\* Significant ( $P < .05$ )

TABLE 17. ANALYSIS OF VARIANCE FOR RUMEN N-BUTYRIC ACID.

Source	D.F.	M.S.	F.	P.
Treatments	2	8.969	4.20	0.063
Blocks	3	1.131	0.53	
Age groups	2	10.013	4.69	
Treatments X blocks	6	7.879	3.69	
Treatments X age groups	4	3.109	1.46	
Remainder	7	2.135		
Total	24			

TABLE 18. ANALYSIS OF VARIANCE FOR RUMEN ISOVALERIC ACID.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.118	0.46	0.647
Blocks	3	0.986	3.87	
Age groups	2	0.458	1.80	
Treatments X blocks	6	0.261	1.02	
Treatments X age groups	2	0.267	1.05	
Remainder	7	0.255		
Total	24			

TABLE 19. ANALYSIS OF VARIANCE FOR RUMEN N-VALERIC ACID.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.034	0.81	0.481
Blocks	3	0.083	1.96	
Age groups	2	0.069	1.64	
Treatments X blocks	6	0.113	2.68	
Treatments X age groups	4	0.246	5.84*	0.022
Remainder	7	0.042		
Total	24			

\* Significant ( $P < .05$ )

TABLE 20. ANALYSIS OF VARIANCE FOR RUMEN ACETIC: PROPIONIC ACIDS RATIO.

Source	D.F.	M.S.	F.	P.
Treatments	2	1.355	0.75	0.506
Blocks	3	1.566	0.87	
Age groups	2	1.231	0.68	
Treatments X blocks	6	0.285	0.16	
Treatments X age groups	4	0.334	0.19	
Remainder	7	1.803		
Total	24			

TABLE 21. ANALYSIS OF VARIANCE FOR RUMEN AMMONIA.

Source	D.F.	M.S.	F.	P.
Treatments	2	2.760	1.23	0.327
Blocks	3	2.416	1.07	
Age groups	2	0.508	0.23	
Treatments X age groups	4	1.766	0.79	
Remainder	12	2.249		
Total	23			

TABLE 22. ANALYSIS OF VARIANCE FOR RUMEN pH.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.076	1.25	0.404
Blocks	3	0.047	0.78	
Age groups	2	0.022	0.35	
Treatments X blocks	6	0.064	1.06	
Treatments X age groups	4	0.009	0.14	
Remainder	1	0.061		
Total	18			

TABLE 23. ANALYSIS OF VARIANCE FOR PACKED CELL VOLUME.

Source	D.F.	M.S.	F.	P.
Treatments	2	7.391	1.33	0.312
Blocks	3	1.436	0.26	
Age groups	2	14.500	2.61	
Treatments X blocks	6	4.639	0.83	
Treatments X age groups	4	13.156	2.36	
Remainder	9	5.645		
Total	26			

TABLE 24. ANALYSIS OF VARIANCE FOR RED BLOOD CELLS.

Source	D.F.	M.S.	F.	P.
Treatments	2	1.128	0.99	0.447
Blocks	3	0.901	0.79	
Age groups	2	4.131	3.63	
Treatments X blocks	6	1.214	1.07	
Remainder	4	1.139		
Total	17			

TABLE 25. ANALYSIS OF VARIANCE FOR WHITE BLOOD CELLS.

Source	D.F.	M.S.	F.	P.
Treatments	2	481.488	16.71*	0.011
Blocks	3	500.302	17.36**	0.009
Age groups	2	61.606	2.14	
Treatments X blocks	6	349.075	12.02*	0.015
Remainder	4	28.813		
Total	17			

\* Significant ( $P < .05$ )\*\* Significant ( $P < .01$ )

TABLE 26. ANALYSIS OF VARIANCE FOR HEMOGLOBIN.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.269	0.36	0.172
Blocks	3	0.063	0.08	
Age groups	2	0.129	0.17	
Treatments X blocks	6	1.187	1.57	
Remainder	4	0.756		
Total	17			



TABLE 27. ANALYSIS OF VARIANCE FOR PLASMA UREA NITROGEN.

Source	D.F.	M.S.	F.	P.
Treatments	2	2.451	0.16	0.852
Blocks	3	34.102	2.28	
Age groups	2	6.086	0.41	
Treatments X blocks	6	14.756	0.99	
Treatments X age groups	4	7.717	0.52	
Remainder	9	14.974		
Total	26			

TABLE 28. ANALYSIS OF VARIANCE FOR PLASMA TOTAL PROTEIN.

Source	D.F.	M.S.	F.	P.
Treatments	2	5.082	1.43	0.289
Blocks	3	2.861	0.81	
Age groups	2	2.001	0.56	
Treatments X blocks	6	3.079	0.87	
Treatments X age groups	4	2.154	0.61	
Remainder	9	3.553		
Total	26			

TABLE 29. ANALYSIS OF VARIANCE FOR PLASMA ALBUMIN.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.215	1.40	0.296
Blocks	3	0.127	0.82	
Age groups	2	0.845	5.50*	0.028
Treatments X blocks	6	0.175	1.14	
Treatments X age groups	4	0.058	0.38	
Remainder	9	0.154		
Total	26			

\* Significant ( $P < .05$ )

TABLE 30. ANALYSIS OF VARIANCE FOR TRIIODOTHYRONINE UPTAKE.

Source	D.F.	M.S.	F.	P.
Treatments	2	2.379	14.27*	0.015
Blocks	3	0.985	5.90	
Age groups	2	0.463	2.78	
Remainder	4	0.167		
Total	11			

\* Significant ( $P < .05$ )

TABLE 31. ANALYSIS OF VARIANCE FOR THYROXINE.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.948	1.22	0.386
Blocks	3	0.043	0.06	
Age groups	2	0.136	0.18	
Remainder	4	0.777		
Total	11			

TABLE 32. ANALYSIS OF VARIANCE FOR FREE THYROXINE INDEX.

Source	D.F.	M.S.	F.	P.
Treatments	2	0.184	1.36	0.354
Blocks	3	0.004	0.03	
Age groups	2	0.017	0.12	
Remainder	4	0.135		
Total	11			