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Effects of Partial Replacement of Berseem Hay by Corn Stalk with or without Calcium Salts of Fish Oil on Rumen Fermentation, Productive and Reproductive Performance of Early Lactating Cows

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Abstract: Ninety early lactating Holstein dairy cows were assigned among 6 groups (15 each) to investigate the effect of dietary partial replacement of Berseem Hay (BH) by Corn Stalk (CS) with or without Calcium Salts of Fish Oil (CaSFO) supplementation on ruminal fermentation characteristics, productive and reproductive performance as well as on some blood serum parameters from 1st week after calving through 12 weeks postpartum. Three experimental diets were formulated without or with CaSFO supplementation to be fed to 6 cows groups (15 per each). Cows fed on 10 or 20% of CS diets loss more body weight, suffering from more negative energy balance and eat less dry matter than control, however, milk-to-feed ratio improved by about 2.5 and 3.8%, respectively compared with control. On the other hand CaSFO supplementation reduced body weight loss, improved feed intake, lessened the negative energy balance and slightly improved milk-to-feed ratio by about 1.3, 0.0 and 0.6%. CS significantly affect ruminal fermentation by production more propionate and less acetate compared with control while, fat supplementation had no significant effect on the rumen fermentation characteristics. CS (10 or 20%) feeding reduced milk production across the experiment by about 1.9 and 5.6%, respectively when compared with control, while milk production improved with fat supplementation. However, either CS or CaSFO supplementation had no significant effect on milk composition. Moreover, CS feeding had no effect ($p > 0.05$) on serum glucose and non-esterified fatty acids at 8th or 12th week postpartum. While CaSFO increased ($p > 0.05$) serum glucose concentration and mostly non significantly reduced NEFA concentration. Also, CS had no effect on the reproductive performance and fat supplementation showed a trend toward improving reproductive performance as indicated by increased percentage of pregnant cows in the first 28 days of breeding. It could be concluded that CS inclusion up to 20% with CaSFO supplementation (11 g kg⁻¹ diet) may offer a potential management practice to improve productive and reproductive performance in dairy cattle.

Key words: Dairy cattle, corn stalk, calcium salt of fish oil, rumen fermentation, productive and reproductive performance

INTRODUCTION

Improvement of livestock production in many developing countries is militated against by a myriad of factors; limiting the increased livestock production on smallholder resource-poor farms in

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these countries is the inadequacy of animal feed resources, especially during the dry season (Lanyasunya *et al.*, 2006). Moreover, the forage supplies are going to be quite limited and in several areas have been reporting unreasonably high hay prices. Obviously, ruminants must have forage in their diets to remain healthy. Also, with the current hay and grain prices, overall feed costs are going to be elevated for quite some time. With these conditions, alternative forages sources are being considered, including the feeding of corn stalk (corn plant after grain harvest). These crop residues are considered low protein content and because of lower starch and higher fiber Corn Stalk (CS) provides less energy than hay (Crowder and Chheda, 1982). Many researchers recommended inclusion of CS in dairy cattle and growing heifers rations with limited levels and some additives (Adams, 2006; Eastridge, 2007; Schroeder, 2008). However, the onset of lactation in the dairy cow is characterized by a dramatic increase in the nutrient demands for milk synthesis that coincides with a prepartum decline in Dry Matter Intake (DMI), this leads to negative energy balance in early lactation (Bell, 1995; Grummer, 1995; Goff and Horst, 1997). Extreme negative energy balance predisposes the cow to the occurrence of several periparturient diseases and health problems that can impact milk production and profitability of the cow during the entire lactation (Grohn *et al.*, 1995; Drackley, 1999). Most approaches to alleviate the period of negative energy balance focus on mitigating the decrease in DMI and include the partial substitution of forages with more energy-dense concentrates and fat supplements (Grummer, 1995). However, high amounts of concentrate may predispose transition cows to excessive decreases in DMI as they approach parturition and potentially increase incidence of displaced abomasum (Coppock *et al.*, 1972; Mashek and Grummer, 2003). In addition, DMI is inversely related to the amount of fat in the diet and is affected by the type of fat and the parity of the animal (Allen, 2000; Hayirli *et al.*, 2002). The utilization of supplemental fat with a more saturated Fatty Acid (FA) profile, such as tallow might have less deleterious effect on rumen fermentation than sources rich in unsaturated FA (Pantoja *et al.*, 1994; Castaneda-Gutierrez *et al.*, 2005; Ben Salem and Bouraoui, 2008). Because microbial activity in the rumen results in lipolysis and biohydrogenation of lipids containing polyunsaturated FA (PUFA) dramatically reduces the amount of PUFA leaving the rumen (Avila *et al.*, 2000; Whitock *et al.*, 2002, 2006). Methods to reduce the susceptibility of FA to such activity are desirable if the intestinal delivery of specific unsaturated FA is to be achieved in ruminants (Carroll *et al.*, 2006). There are different commercial sources of rumen inert fats including hydrogenated FA and Calcium Salt (CaS) FA. These fat sources were originally designed to increase the caloric intake of dairy cows with minimal impact on rumen microbial activity and milk production (Juchem *et al.*, 2007).

Moreover, pregnancy rate is important determinant of profitability in dairy operations. High herd pregnancy rate increases the lifetime milk production of cows and reduces the costs associated with Artificial Insemination (AI) and replacement animals. Fish oil is relatively high concentrations of two PUFA of the n-3 family, eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA) and have been reported to be 10.8 and 11.1% of total fatty acids (Donovan *et al.*, 2000). Feeding EPA and DHA may prevent regression of the CL and increase fertility rates (Staples *et al.*, 1998; Mattos *et al.*, 2002; Ben Salem and Bouraoui, 2008).

The current study was designed to evaluate the effect of partial replacement of BH by CS with or without dietary EPA and DHA supplementation (from fish oil incorporated into calcium salt) on dry matter intake, rumen fermentation characteristics, productive and reproductive performance of dairy cows during early stage of lactation.

MATERIALS AND METHODS

This experiment was carried out at Al-Mashary Dairy Farm in the middle area of Saudi Arabia (started at mid march 2008 to the second half of June 2008) to evaluate the partial replacement of

Berseem Hay (BH) by Corn Stalk (CS) without or with rumen protected fish oil supplementation on rumen fermentation characteristics, productive and reproductive performance of early dairy cows.

Cows, Experimental Design and Diets

Ninety early lactating Holstein dairy cows were allotted among 6 groups (15 cows per each). The first group (15 cows) was assigned to the control group without corn stalk or fat supplementation. TMR to meet the predicted requirements for energy, protein, minerals and vitamins according to NRC (2001) were formulated (basal diet No.1) without fat supplementation and fed to group No. 1 (control). BH was replaced by CS (v/v) at 25 and 50% from the hay quantity of the basal diet and the diet adjusted accordingly to be iso-nitrogenous and fed for groups 2 and 3, respectively. Calcium Salt of Fish Oil (CaSFO) was supplied from commercial product produced by Centraly's Co., (Progress ferti +) at (11 g kg⁻¹) diet to increase the caloric density of the diet. The experimental period started from the 1st day postpartum through 12 weeks of lactation.

The housing system provides (0.45 cm) head lock for feeding and about 30 m² yard per cow. The barns have open ridges to facilitate cleaning and manure removing. About 30% of the whole area is sloppy shaded at height (9 m) in the center with fan but no available cooling system in the cow's yard. Waterers were available in suitable size all the day time. Cows were fed *ad libitum* on a total mixed ration. Ingredient and chemical compositions of the used diet are presented in Table 1 and 2, respectively.

Feed Intake and Body Weight

Diets were offered in equal amounts twice daily (08:00 and 15:00). Feed consumption was recorded daily by weighing feeds offered to and refused per each group. Samples of Total Mixed Ration (TMR) and feed ingredients were collected daily and kept frozen. Samples were composite by period (each 4 weeks), dried at 55°C for 48 h, ground through a 1 mm screen (Wiley mill) and analyzed for DM, total nitrogen, NDF, ADF, EE composition. Body Weight (BW) was determined 1 day postpartum and then each 4 weeks after the am milking. Based on that milk-to-feed ratio and net energy balance for postpartum period were calculated monthly (NRC, 2001).

Table 1: Physical composition of the used postpartum diets

| Ingredients | Diet number and % of corn stalk | | | | | |
|----------------------------|---------------------------------|------------|---------------|------------|---------------|------------|
| | 1(0.0%) | | 2(10%) | | 3(20%) | |
| | Without CaSFO | With CaSFO | Without CaSFO | With CaSFO | Without CaSFO | With CaSFO |
| Berseem hay | 46.00 | 46.00 | 34.50 | 34.50 | 23.00 | 23.00 |
| Corn stalk | 0.00 | 0.00 | 11.50 | 11.50 | 23.00 | 23.00 |
| Yellow corn grain | 23.90 | 22.80 | 21.40 | 20.30 | 18.90 | 17.80 |
| Barley grain | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Soybean meal | 7.00 | 7.00 | 9.50 | 9.50 | 12.00 | 12.00 |
| Cotton seed | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Sugar beet pulp | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 | 1.75 |
| NaCl (Iodized) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Lime stone | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Dicalcium phosphate | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Sodium bicarbonate | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Calcium salt of fish oil** | 0.00 | 1.10 | 0.00 | 1.10 | 0.00 | 1.10 |
| Mineral and vitamin pr.*** | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Molasses | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |

Calcium Salt of Fish Oil (CaSFO) Progress Ferti + product produced by Centraly's Co., (France) progress Ferti+a special form of calcium salt fish oil, which has min. 80% fish oil, 12% calcium, palmitic acid 23%, stearic acid 5%, oleic 12%, EPA 8% and DHA 10% * Cattle premix produced by Centraly's Co., (France) contains the following elements per kg (10000000 IU vitamin A, 1000000 IU vit. D3, 10000 mg vit. E, 100000 mg magnesium, 50000 mg manganese, 45000 mg zinc, 50000 mg iron, 6000 mg copper, 800 mg iodine, 100 mg selenium)

Table 2: Chemical composition of the used diets

| Ingredients | Diet number and % of corn stalk | | | | | |
|-------------------------------|---------------------------------|------------|---------------|------------|---------------|------------|
| | 1(0.0%) | | 2(10%) | | 3(20%) | |
| | Without CaSFO | With CaSFO | Without CaSFO | With CaSFO | Without CaSFO | With CaSFO |
| Crude protein (%) | 16.82 | 16.75 | 16.60 | 16.60 | 16.55 | 16.60 |
| Ether extract (%) | 2.90 | 3.80 | 2.70 | 3.60 | 2.50 | 3.40 |
| Crude fiber (%) | 14.50 | 14.70 | 16.40 | 16.30 | 18.30 | 18.40 |
| NDF (%) | 26.80 | 26.70 | 30.40 | 30.40 | 34.00 | 33.90 |
| ADF (%) | 18.40 | 18.50 | 20.40 | 20.30 | 22.30 | 22.40 |
| NFC (%)* | 46.00 | 45.20 | 43.30 | 42.40 | 40.50 | 39.70 |
| Calcium (%) | 1.20 | 1.30 | 1.00 | 1.20 | 0.90 | 1.00 |
| Phosphorus (%) | 0.50 | 0.48 | 0.50 | 0.49 | 0.48 | 0.50 |
| NE _t (Mcal/kg DM)* | 1.56 | 1.62 | 1.51 | 1.58 | 1.47 | 1.54 |

*Calculated according to NRC (2001)

Milk Production and Milk Composition

Cows were milked twice daily in the milking paller (05:00 and 17:00) and milk yield was recorded at each milking. During the last week of each 28 days period, milk samples were taken from each cow at each milking, pooled on a yield basis and stored at +4°C with a preservative (bronopol-B2) until analyzed for fat, protein and lactose.

Ruminal Fermentation Characteristics

Ruminal fluids were collected from 5 dairy cows at 0, 4 and 8 h after feeding from 5 cows from each group on 28, 56 and 84 days during the experiment. Ruminal fluids collected through a speculum were inserted into the cow mouth and a lubricated rubber tube was inserted through the speculum into the rumen via the esophagus. Ruminal contents (250 mL) were removed using an electric pump. Samples were monitored visually to ensure they were not contaminated with saliva. The pH was measured immediately using pH meter (Orion research model 201). The whole contents were squeezed through 4 layers of cheesecloth. The samples were acidified to pH 2 with 50% H₂SO₄ and frozen at -20°C for later determination of Volatile Fatty Acids (VFA) concentrations.

Blood Sampling

Five cows from each treatment group were randomly selected for blood sampling (the animals selected are the same for rumen fluids collection). Blood samples were collected at 8 and 12 weeks after calving. Blood samples were obtained by vein puncture during or immediately after evening milking. Blood was collected into a (20 mL) tube and allowed to clot at ambient temperature. Blood samples were centrifuged at 3000 rpm for 15 min. Only clear non-hemolyzed sera were obtained and kept frozen until further analysis. Samples were analyzed for glucose according to Trinder (1969). Serum samples were used for the NEFA assay, following the procedures from the NEFA C kit by Wako Chemicals USA, Inc. (ACS-ACOD method), Richmond, VA the NEFA assay was performed on one day, with an intra assay-coefficient of variation of 2.63%.

Chemical Analysis

Analytical DM contents of TMR, feed ingredients and feces were determined by oven-drying at 105°C for 48 h (AOAC, 1990; method 930.15). Ash contents of TMR, feed ingredients and feces were determined by incineration at 550°C overnight.

Crude protein in TMR and feed ingredient were determined by using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963). NDF and ADF in TMR and feed ingredients were

determined according to AOAC (1990) method. Protein, fat and lactose concentrations in milk samples were analyzed AOAC (1990) by infrared spectrophotometer (System 6000 MilkoScan; Foss Electric). Concentrations of VFA in ruminal fluid were analyzed by calorimetric (Weatherburn, 1967) and by GLC (Varian 3700; Varian Specialties Ltd., Brockville, Ontario, Canada).

Reproduction Data

Reproductive management was done in accordance with standard operating procedures of the dairy farms. At seven days before the planned breeding period, cows that were not observed to be in estrus by dairy personal following 3 weeks of estrus detection were checked by the veterinarian to determine the anestrus % of each group. All cows were bred upon estrus detection by artificial insemination. Cows were checked for pregnancy using rectal palpation to assess pregnancy rates in the first 28 and 44 days of the breeding time. Cows were rechecked again 6 weeks later to determine pregnancy rates in the first 60 days of breeding and confirm final pregnancy rates.

Statistical Analysis

Data were analyzed by the General Linear Model (GLM) procedure (SAS, 1996). The Least Square Mean (LSM)+Standard Errors (SE) were calculated and tested for significance using the t-test (Steel and Torrie, 1960).

RESULTS

Body Weight Changes and Dry Matter Intake

Cows fed on Corn Stalk (CS) containing diets (10 or 20%) loss ($p>0.05$) more body weight (Table 3) during the 1st, 2nd and 3rd month of the postpartum period by about 4.2 and 4.9%, 2 and 2% and 1.85 and 2.25%, respectively versus 3.4, 1.2 and 0.7% for the control group. Dairy cows fed on the diet containing CS (10 or 20%) loss more body weight than control during across the experimental period (7.85 and 8.8 vs. 5.2%), respectively. Fat supplementation (CaSFO) reduced body weight loss (1.0, 4.1 and 4.9 vs. 5.2, 7.8 and 8.8%), respectively for control and groups fed on 10 or 20% of CS with and without CaSFO supplementation.

CS inclusion at 10 or 20% levels in dairy cow diet reduced ($p<0.05$) Dry Matter Intake (DMI) during the 1st and 3rd month postpartum (Table 4) by about 6.8 and 12.5% and 5.9 and 6.8%, respectively when compared with control. CS inclusion 10 or 20% in dairy cow diet reduced DMI

Table 3: Effect of berseem hay replacement by com stalk without or with rumen protected fish oil supplementation on postpartum body weight changes of dairy cows

| Stages of lactation | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation (n = 15) | With rumen protected fish oil supplementation (n = 15) |
|---------------------|------------------------------------|---|--|
| One day postpartum | 0.0 | 593±3.4 ^{ax} | 588±2.9 ^{ax} |
| | 10.0 | 589±3.8 ^{ax} | 586±3.7 ^{ax} |
| | 20.0 | 591±2.9 ^{ax} | 590±3.1 ^{ax} |
| 4 weeks postpartum | 0.0 | 573±3.7 ^{ax} | 584±3.9 ^{ax} |
| | 10.0 | 564±4.3 ^{ax} | 573±3.2 ^{abx} |
| | 20.0 | 562±3.9 ^{ax} | 570±3.7 ^{bx} |
| 8 weeks postpartum | 0.0 | 566±2.8 ^{ax} | 580±3.0 ^{ax} |
| | 10.0 | 553±3.3 ^{abx} | 568±3.1 ^{bx} |
| | 20.0 | 551±2.8 ^{ax} | 564±2.9 ^{bx} |
| 12 weeks postpartum | 0.0 | 562±4.1 ^{ax} | 582±3.7 ^{ay} |
| | 10.0 | 543±3.8 ^{ay} | 562±3.9 ^{bx} |
| | 20.0 | 539±3.2 ^{bx} | 561±3.8 ^{bx} |

Values are expressed as Mean±SE. Mean values with different letter(s) at the same column (a-d letters) or row (x-y letters) and period differ significantly at $p\leq 0.05$

Table 4: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on Dry Matter Intake (DMI) of dairy cows

| Stages of lactation | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation | | With rumen protected fish oil supplementation | |
|-----------------------|------------------------------------|--|---------------------------|---|---------------------------|
| | | kg day ⁻¹ | % from BW | kg day ⁻¹ | % from BW |
| 0-4 weeks postpartum | 0.0 | 17.77±0.15 ^{ax} | 3.05±0.007 ^{ax} | 18.92±0.12 ^{ax} | 3.13±0.00 ^{ax} |
| | 10.0 | 16.56±0.18 ^{abx} | 2.87±0.012 ^{bx} | 17.71±0.16 ^{abx} | 3.06±0.013 ^{abx} |
| | 20.0 | 15.54±0.23 ^{bx} | 2.70±0.028 ^{cx} | 17.27±0.18 ^{ax} | 2.98±0.015 ^{bx} |
| 5-8 weeks postpartum | 0.0 | 20.43±0.43 ^{xy} | 3.59±0.074 ^{xy} | 21.25±0.41 ^{xy} | 3.65±0.066 ^{xy} |
| | 10.0 | 19.46±0.35 ^{aby} | 3.48±0.054 ^{aby} | 20.67±0.45 ^{xy} | 3.62±0.075 ^{xy} |
| | 20.0 | 18.91±0.38 ^{xy} | 3.40±0.065 ^{by} | 20.24±0.38 ^{xy} | 3.57±0.061 ^{xy} |
| 9-12 weeks postpartum | 0.0 | 22.55±0.49 ^{yz} | 4.01±0.074 ^{yz} | 23.98±0.38 ^{yz} | 4.18±0.049 ^{yz} |
| | 10.0 | 21.22±0.44 ^{yz} | 3.87±0.069 ^{yz} | 23.21±0.46 ^{yz} | 4.12±0.067 ^{yz} |
| | 20.0 | 21.01±0.56 ^{yz} | 3.86±0.100 ^{yz} | 22.15±0.55 ^{yz} | 3.95±0.091 ^{yz} |
| Mean | 0.0 | 20.25 | 3.55 | 21.18 | 3.65 |
| | 10.0 | 19.08 | 3.41 | 20.53 | 3.60 |
| | 20.0 | 18.49 | 3.32 | 19.89 | 3.50 |

Values are expressed as Mean±SE. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at p≤0.05

Table 5: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on rumen fermentation characteristics in dairy cows

| Items | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation | With rumen protected fish oil supplementation |
|---------------------------|------------------------------------|--|---|
| | | (n = 15) | (n = 15) |
| pH | 0.0 | 6.22±0.04 ^{ax} | 6.29±0.06 ^{ax} |
| | 10.0 | 6.39±0.05 ^{ax} | 6.32±0.4 ^{ax} |
| | 20.0 | 6.29±0.05 ^{ax} | 6.29±0.3 ^{ax} |
| Total VFA (mM) | 0.0 | 92.3±1.3 ^{ax} | 93.6±1.2 ^{ax} |
| | 10.0 | 94.3±1.5 ^{ax} | 96.4±1.6 ^{ax} |
| | 20.0 | 96.5±1.4 ^{ax} | 98.6±1.3 ^{ax} |
| Acetate (A) mol/100 mL | 0.0 | 64.3±0.5 ^{ax} | 63.5±0.4 ^{ax} |
| | 10.0 | 62.4±0.6 ^{abx} | 61.6±0.6 ^{abx} |
| | 20.0 | 61.2±0.7 ^{bx} | 61.2±0.6 ^{ax} |
| Propionate (P) mol/100 mL | 0.0 | 20.9±0.6 ^{ax} | 22.5±0.5 ^{ax} |
| | 10.0 | 23.6±0.7 ^{bx} | 25.1±0.9 ^{bx} |
| | 20.0 | 25.3±0.7 ^{bx} | 26.6±0.8 ^{bx} |
| Butyrate mol/100 mL | 0.0 | 14.8±0.8 ^{ax} | 14.0±0.5 ^{ax} |
| | 10.0 | 14.0±0.5 ^{ax} | 13.3±0.7 ^{ax} |
| | 20.0 | 13.5±0.6 ^{ax} | 12.2±0.9 ^{ax} |
| A:P ratio | 0.0 | 3.08±0.084 ^{ax} | 2.82±0.05 ^{ay} |
| | 10.0 | 2.64±0.069 ^{bx} | 2.45±0.082 ^{bx} |
| | 20.0 | 2.42±0.051 ^{cx} | 2.30±0.061 ^{bx} |

Values are expressed as Mean±SE. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at p≤0.05

across the experiment by about 5.8 and 8.7%, respectively when compared with the control. CaSFO supplementation improved (p>0.05) DMI throughout the experiment by about 4.6, 7.65 and 7.6% when compared with cow group fed on the same diet without CaSFO supplementation.

Rumen Fermentation Characteristics

CS inclusion (10 or 20%) instead of BH on dairy cows diet increased (p>0.05) ruminal pH (Table 5) by about 2.7 and 1.1%, respectively. CS addition instead of BH in dairy cows diet increased (p>0.05) total volatile fatty acids, decreased acetate and in contrast increased (p<0.05) propionate and significantly (p<0.05) decreased acetate to propionate ratio when compared with the control. Moreover, protected fish oil supplementation had no significant effect on ruminal fermentation characteristics when compared with cows group fed on the same diet without oil supplementation.

Table 6: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on postpartum milk production (kg/cow/day) of dairy cows

| Items | Inclusion levels of corn stalk (%) | Without rumen protected | With rumen protected |
|-----------------------|------------------------------------|-----------------------------------|-----------------------------------|
| | | fish oil supplementation (n = 15) | fish oil supplementation (n = 15) |
| 0-4 weeks postpartum | 0.0 | 29.5±0.56 ^{ax} | 31.3±0.66 ^{ax} |
| | 10.0 | 28.4±0.45 ^{abx} | 30.4±0.53 ^{ax} |
| | 20.0 | 27.3±0.56 ^{bx} | 30.1±0.43 ^{ax} |
| 5-8 weeks postpartum | 0.0 | 32.9±0.43 ^{ay} | 34.8±0.51 ^{ay} |
| | 10.0 | 31.5±0.81 ^{ay} | 33.9±0.49 ^{ay} |
| | 20.0 | 30.8±0.72 ^{ay} | 33.2±0.70 ^{ay} |
| 9-12 weeks postpartum | 0.0 | 34.4±0.65 ^{ay} | 37.7±0.73 ^{az} |
| | 10.0 | 33.9±0.62 ^{az} | 36.3±0.45 ^{az} |
| | 20.0 | 33.3±0.74 ^{az} | 35.8±0.53 ^{az} |
| Mean | 0.0 | 32.3 | 34.6 |
| | 10.0 | 31.6 | 33.5 |
| | 20.0 | 30.5 | 33.0 |

Values are expressed as Mean±SE. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at p≤0.05)

Table 7: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on milk composition of dairy cows

| Stages of location | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation | | | With rumen protected fish oil supplementation | | |
|---------------------------------------|------------------------------------|--|------------------|------------------|---|------------------|------------------|
| | | Milk fat (%) | Milk protein (%) | Milk lactose (%) | Milk fat (%) | Milk protein (%) | Milk lactose (%) |
| 0-4 weeks postpartum | 0.0 | 3.35±0.04 | 3.12±0.02 | 4.65±0.14 | 3.31±0.05 | 3.22±0.06 | 4.55±0.13 |
| | 10.0 | 3.25±0.05 | 3.21±0.02 | 4.55±0.15 | 3.27±0.03 | 3.29±0.05 | 4.45±0.12 |
| | 20.0 | 3.19±0.05 | 3.25±0.03 | 4.49±0.15 | 3.23±0.04 | 3.30±0.04 | 4.49±0.16 |
| 5-8 weeks postpartum | 0.0 | 3.15±0.04 | 3.09±0.06 | 4.55±0.14 | 3.23±0.04 | 3.15±0.04 | 4.45±0.16 |
| | 10.0 | 3.12±0.03 | 3.15±0.03 | 4.35±0.15 | 3.25±0.02 | 3.28±0.03 | 4.39±0.09 |
| | 20.0 | 3.11±0.03 | 3.18±0.05 | 4.59±0.15 | 3.18±0.03 | 3.33±0.06 | 4.46±0.11 |
| 9-12 weeks postpartum | 0.0 | 3.09±0.03 | 3.15±0.04 | 4.45±0.14 | 3.12±0.04 | 3.23±0.04 | 4.54±0.14 |
| | 10.0 | 3.05±0.04 | 3.21±0.04 | 4.35±0.15 | 3.21±0.04 | 3.30±0.03 | 4.57±0.16 |
| | 20.0 | 3.06±0.05 | 3.22±0.05 | 4.39±0.15 | 3.15±0.03 | 3.26±0.03 | 4.48±0.15 |
| Average (%) | 0.0 | 3.20 | 3.12 | 4.55 | 3.22 | 3.20 | 4.51 |
| | 10.0 | 3.14 | 3.19 | 4.42 | 3.24 | 3.29 | 4.47 |
| | 20.0 | 3.12 | 3.22 | 4.49 | 3.19 | 3.30 | 4.48 |
| Average yield (kg day ⁻¹) | 0.0 | 1.034 | 1.008 | 1.47 | 1.114 | 1.107 | 1.56 |
| | 10.0 | 0.992 | 1.008 | 1.39 | 1.085 | 1.102 | 1.497 |
| | 20.0 | 0.952 | 0.982 | 1.37 | 1.053 | 1.089 | 1.478 |

Values are expressed as Mean±SE. No significant differences p>0.05

Milk Production and Composition

Reduction (p>0.05) of milk yield was observed with CS inclusion at 10% of the dairy cow diet (Table 6) during the 1st month of lactation by about 3.7% when compared with the control, while CS inclusion at higher level (20%) significantly (p<0.05) decreased milk yield by about 7.5% during the same period. However, the reduction of milk production by the cow groups fed on CS (10 or 20%) in their diet was non significantly (p>0.05) during the 2nd and 3rd month of lactation. Reduction of milk yield was observed with CS addition (10 or 20%) across the experiment by about 1.9 and 5.6%, respectively when compared with the control. CaSFO supplementation increased (p>0.05) milk yield by about 6.1, 7 and 10.3%; 5.8, 7.6 and 7.8% and 9.6, 7.1 and 7.5% during the 1st, 2nd and 3rd month of lactation, respectively when compared with the production of cows group fed on the same diet without CaSFO supplementation, while milk yield improvement throughout the experiment was observed with fat supplementation by about 7.1, 6 and 8.2.

Inclusion of CS in dairy cattle diets instead of BH with or without CaSFO supplementation had no effect (p>0.05) on milk fat, protein and lactose % (Table 7). However, milk fat yield (kg day⁻¹)

Table 8: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on feed conversion efficiency and Net Energy Balance (NEB) of dairy cows

| Stages of lactation | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation | | With rumen protected fish oil supplementation | |
|-----------------------|------------------------------------|--|-----------------|---|-----------------|
| | | Milk to feed ratio | Postpartum NEB* | Milk to feed ratio | Postpartum NEB* |
| 0-4 weeks postpartum | 0.0 | 1.66±0.05 ^{ax} | -3.52 | 1.65±0.069 ^{ax} | -1.95 |
| | 10.0 | 1.71±0.03 ^{bx} | -4.95 | 1.72±0.04 ^{bx} | -3.97 |
| | 20.0 | 1.76±0.03 ^{cx} | -6.09 | 1.74±0.018 ^{cx} | -4.63 |
| 5-8 weeks postpartum | 0.0 | 1.61±0.04 ^{ay} | -0.71 | 1.64±0.02 ^{ax} | 0.09 |
| | 10.0 | 1.62±0.03 ^{ay} | -1.82 | 1.64±0.03 ^{ay} | -1.41 |
| | 20.0 | 1.63±0.02 ^{ay} | -3.05 | 1.64±0.01 ^{ay} | -1.82 |
| 9-12 weeks postpartum | 0.0 | 1.53±0.01 ^{az} | 1.98 | 1.57±0.015 ^{ay} | 2.66 |
| | 10.0 | 1.59±0.01 ^{by} | -0.40 | 1.56±0.03 ^{az} | 1.05 |
| | 20.0 | 1.58±0.02 ^{bz} | -0.93 | 1.62±0.04 ^{by} | -0.44 |
| Mean | 0.0 | 1.60 | -0.75 | 1.62 | 0.27 |
| | 10.0 | 1.64 | -2.39 | 1.64 | -1.43 |
| | 20.0 | 1.66 | -3.36 | 1.67 | -2.30 |

Values are expressed as Mean±SE. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at $p \leq 0.05$. * Net Energy Balance (calculated based on NRC, 2001) = Dry matter intake × NE_L content of diet – {(0.08 × body weight^{0.75}) + ((0.0929 × fat (%) + 0.0563 × protein (%) + 0.0395 × lactose (%) × milk yield)}

decreased by about 4.1 and 7.9% by cows fed on diet containing 10 or 20% of CS, respectively when compared with control, while protein yield nearly similar. Moreover, fat supplementation increased fat, protein and lactose yield (kg day⁻¹) by about 7.7, 9.4 and 10.6%; 9.8, 9.3 and 10.9% and 6.1, 7.7 and 7.9%, respectively when compared with cow group fed on the same diet without fat supplementation.

Feed Efficiency

CS addition (10 or 20%) in dairy cattle diet improved ($p < 0.05$) milk-to-feed ratio (Table 8) during the 1st and 3rd month of lactation by about (3 and 6%) and (3.9 and 3.3%), respectively while, the improvement was insignificant during the 2nd month of lactation when compared with the control. Moreover, CS addition (10 or 20%) improved milk-to-feed ratio across the experimental period by about 2.5 and 3.8%, respectively when compared with control. CaSFO supplementation slightly improved milk-to-feed ratio by about 1.3, 0.0 and 0.6% across the experiment when compared with cows group fed on the same diet without fat supplementation.

Cows fed on the basal diet (no CS addition) were in a less negative net energy balance across the experimental period than cows fed on diet containing 10 or 20% of CS by about 218.7 and 348%, respectively, while CaSFO supplementation improved postpartum net energy balance across the experiment by about 36, 40.2 and 31.5% when compared with cows group fed on the same diet without fat supplementation.

Blood Serum Constituents

CS addition (10 or 20%) in dairy cattle diet increased ($p > 0.05$) serum glucose concentration at 8th and 12th weeks postpartum (Table 9) by about (1.8 and 2.9%) and (1.5 and 2.4%), respectively when compared with control. Moreover, CaSFO supplementation increased ($p > 0.05$) serum glucose when compared with cattle group fed on the same diet without CaSFO supplementation.

No significant difference in NEFA serum concentration between cow groups fed on diet containing CS and the control (Table 9). While, CaSFO supplementation reduced ($p < 0.05$) NEFA serum concentration in cows fed on the basal diet without CS addition by about 18.3 and 35.8% at 8th and 12th week postpartum when compared with cows group fed on the diet without oil supplementation. However, CaSFO within the diet containing CS mostly had no effect ($p > 0.05$) on blood serum NEFA concentration compared with the groups fed on the same diet without oil supplementation.

Table 9: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on some blood parameters of dairy cows

| Items | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation | | With rumen protected fish oil supplementation | |
|--------------------------------|------------------------------------|--|-------------------------|---|-------------------------|
| | | 8 weeks postpartum | 12 weeks postpartum | 8 weeks postpartum | 12 weeks postpartum |
| Glucose (mg dL ⁻¹) | 0.0 | 55.5±0.92 ^{ac} | 58.4±0.76 ^{bc} | 56.7±0.88 ^{ac} | 59.6±0.79 ^{bc} |
| | 10.0 | 56.6±0.78 ^{ac} | 59.3±0.96 ^{bc} | 57.7±0.94 ^{ac} | 60.2±1.03 ^{bc} |
| | 20.0 | 57.1±0.82 ^{ac} | 59.8±0.68 ^{bc} | 57.8±0.85 ^{ac} | 61.1±1.01 ^{bc} |
| NEFA (mEq L ⁻¹) | 0.0 | 0.82±0.05 ^c | 0.53±0.04 ^d | 0.67±0.05 ^d | 0.34±0.08 ^e |
| | 10.0 | 0.79±0.03 ^c | 0.49±0.03 ^d | 0.73±0.06 ^c | 0.41±0.06 ^d |
| | 20.0 | 0.84±0.05 ^c | 0.48±0.04 ^d | 0.71±0.07 ^c | 0.39±0.07 ^e |

Values are expressed as Means±SE. Mean values with different letters at the same column (a - d letters) or row (x-y letters) and period differ significantly at p≤0.05

Table 10: Effect of berseem hay replacement by corn stalk without or with rumen protected fish oil supplementation on reproductive performance of dairy cows

| Items (%) | Inclusion levels of corn stalk (%) | Without rumen protected fish oil supplementation (n = 15) | With rumen protected fish oil supplementation (n = 15) |
|-----------------------|------------------------------------|---|--|
| Anestrous cows* | 0.0 | 33.3 | 26.7 |
| | 10.0 | 33.3 | 26.7 |
| | 20.0 | 26.7 | 26.7 |
| 28 day pregnancy rate | 0.0 | 40.0 | 53.3 |
| | 10.0 | 33.3 | 53.3 |
| | 20.0 | 33.3 | 46.7 |
| 49 day pregnancy rate | 0.0 | 46.6 | 60.0 |
| | 10.0 | 40.0 | 53.3 |
| | 20.0 | 40.0 | 53.3 |
| 60 day pregnancy rate | 0.0 | 60.0 | 73.3 |
| | 10.0 | 60.0 | 73.3 |
| | 20.0 | 53.3 | 66.7 |

Values are expressed as Means±SE. Mean values with different letters at the same column (a-d letters) or row (x-y letters) and period differ significantly at p≤0.05; *Cows visually observed by dairy person to be non-cycling 7 days before planned start of mating

Reproductive Performance

CS inclusion (10 or 20) in dairy cows diet decreased 49 and 60 days pregnancy rate (Table 10) by about 14.2 and 14.2% and 0.0 and 11.25, respectively when compared with control, while fat supplementation improved pregnancy rate % of dairy cows at 49 and 60 days postpartum by about 28.8, 33.3 and 33.3% and 22.2, 22.2 and 25.1%, respectively when compared with cows group fed on the same diet without CaSFO supplementation.

DISCUSSION

Body Weight Changes and Dry Matter Intake

The observed reduction of body weight losses of dairy cows fed on diets supplemented by CaSFO may be related to increased energy density of the diet and this results are in harmony with those obtained by Sklan *et al.* (1991) and Ben Salem and Bouraoui (2008) where they indicated that body condition scores were higher for cows fed diets supplemented with fat and fat plus methionine compared with the control. DMI reduction with CS feeding may be related to the higher NDF of the diet containing CS which increased NDF from 26.8% of the control to 30.4 and 34.0% in the diet containing 10 and 20% of the used alternative roughage. The present data are supported by those obtained by Ruiz *et al.* (1995) who reported that increased dietary NDF concentration through feeding bermuda grass and whole corn plant linearly decreased DMI of lactating cows Zebeli *et al.* (2008) who stated that feeding dairy cows on diets containing higher NDF (31.9±1.97%) slightly decreased DMI

compared with the lower levels. Also, Arelovich *et al.* (2008) indicated that total DM and NE_L intakes decreased with increasing dietary NDF. The data are in harmony with the data obtained by Eastridge (2007) indicated that as the amount of corn stover pellet increased in the dairy cattle diet, DMI decreased. Moreover, the lower DMI of animal groups fed on diet containing different levels of corn stalk may explain the higher body weight losses. The present data are in contrast by Son *et al.* (1996) and Been Salem and Bouraoui (2008) the difference may be due to the different of fat sources and the using of rumen protected oil rather than free oil in the diets. On the other hand the response of fat supplementation seems to be depend on the type of forage fed (Ruppert *et al.*, 2003; Onetti *et al.*, 2004). The data suggest that interaction of fat and fiber sources is an important determinant of response to fat sources. These explanations supported by the data obtained by Juchem *et al.* (2007). Moreover, the data suggest that fish oil protection by calcium salt minimized the negative effect of fish oil when supplemented unprotected on rumen fermentation which reflected markedly on DMI suppression (Castaneda-Gutierrez *et al.*, 2005). DMI improvements with CaSFO supplementation provided more energy for the animals and minimize the body weight loss when compared with dairy cows groups fed on the same diet without fat supplementation.

Rumen Fermentation Characteristics

In regard to ruminal pH, it was observed that CS addition instead of BH increased pH which may be related to the higher NDF% of the diet and consequently stabilize ruminal pH and reduce the risk of acidosis and the high fibrous content improve propionic acid production. The present data are in agreement with those obtained by Zebeli *et al.* (2008), who they stated that the level of about 30-33% of NDF in the dairy cattle diet may be considered generally optimal for minimizing the risk of subclinical ruminal acidosis. Moreover, because of the fish oil used in this study in the form of calcium salt (rumen inert product) it was expected that feeding CaSFO would cause no significant effect of rumen pH or fermentation characteristics compared with the cows group fed on the same diet without oil supplementation. The data are in harmony with those obtained by Juchem *et al.* (2007) who stated that the characteristics of CaSFO would qualify it as an appropriate dietary fat supplements to replace tallow in dairy cattle ration and alleviate any negative effect of PUFA on rumen fermentation and improve the incorporation of PUFA in the animal products. Also, Huang *et al.* (2008) suggested that fat supplements had no effect on rumen fermentation.

Milk Production and Composition

Milk yield reduction with CS addition instead of BH in dairy cattle ration and the possible explanation may be related to the lower DMI and less energy content. The present results are supported by Eastridge (2007) who stated that milk yield decreased as the amount of corn stover increased in dairy cattle diet. Moreover, lowered milk production with CS addition may be related to the higher fiber and NDF percent of the diet and that explanation supported by Ruiz *et al.* (1995) and Zebeli *et al.* (2008) indicated that feeding diets with NDF 31.9±1.97% slightly decreased actual milk yield. On the other hand improvement of milk yield with CaSFO supplementation may be related to the more caloric effect and increased DMI. These results are in agreement with those obtained by Ben Salmen and Bouraoui (2008) who stated that calcium salt of palm oil supplementation increased milk yield compared with control group. Also calcium salts of palm oil increased milk production in some studies (Scott *et al.*, 1995; Harrison *et al.*, 1995). Although those positive results have not always been consistent throughout the lactation (Bojalil *et al.*, 1998). Also, more recent study indicated that milk yield was similar for cows fed calcium salt of fish oil and tallow (Juchem *et al.*, 2007). The difference between studies may be related to sources of used roughage, nutrient digestibility and fat sources. Moreover, these data suggest that interaction of fat and fiber source is an important factor and inclusion of low energy roughage source into a diet improve production response to fat sources that are considered inert in the rumen.

The slight depression of milk fat% and improvement of milk protein% with CS addition instead of BH in dairy cows diet may be related to the decreased molar (acetate to propionate ratio) of the rumen fermentation. An improvement of milk fat, protein and lactose percent and yield with CaSFO supplementation resulted from high energy intake and more milk yield. The present results are supported by Bernal-Santos *et al.* (2003) who reported that supplementation of Conjugated Linoleic Acid (CLA) in early lactation cows have an increase in milk and milk protein yields. Also, Ben Salmen and Bouraoui (2008) observed an increase ($p < 0.005$) in milk fat and protein yield (kg day^{-1}) with fat supplementation when compared with control. However, this is not always observed as milk protein and lactose yield were not affected by fat supplementation during early lactation (Moore *et al.*, 2004; Selberg *et al.*, 2004) and the differences related to the fat sources and the authors used unprotected oil which lead to DMI depression and negatively modified rumen fermentation.

Feed Efficiency

In regard to milk-to-feed ratio was improved with CS inclusion instead of BH in dairy cows diet and that may be due to related to lower DMI but at the same time the cows were in more negative energy balance and loss more body which counteracted by CaSFO supplementation which may actually be decreasing net lipolysis, allowing an increased feed intake and reducing the use of body reserves in early lactation. This is supported by the data obtained by Juchem *et al.* (2007).

Blood Serum Constituents

Non significant improved serum glucose concentration with CS addition instead of BH may be related to higher rumen propionate production and the results are supported by earlier studies observed no significant effect of CLA on plasma concentrations of glucose and insulin (Bernal-Santos *et al.*, 2003; Selberg *et al.*, 2004). Moreover, CaSFO slightly increased blood serum glucose in dairy cows when compared with cows fed on the same diet without CaSFO supplementation this may be related to the fish oil reduced the insulin response to glucose (Pighin *et al.*, 2003). CS addition non had no effect on serum NEFA Concentration, while CaSFO supplementation reduced NEFA which explain the less body loss of the cows fed on diet containing fat. Also, NEFA can reflect the degree of adipose tissue mobilization, but might also be reflected by the supply of fatty acid to the small intestine (Drackley *et al.*, 1992). A reduced NEFA concentration was previously observed with Castaneda-Gutierrez *et al.* (2005) where they reported that cows group supplemented with CLA tended to have lower plasma NEFA concentration during the first 9 weeks of lactation. Also, Ballou *et al.* (2009) reported that dietary supplemental fish oil tended to increase plasma glucose and decreased NEFA during the postpartum period. In contrast other studies in early and established lactation have not observed differences in NEFA caused by treatment with CLA (Bernal-Santos *et al.*, 2003; Moore *et al.*, 2004) which used another fat sources and different ration ingredients.

Reproductive Performance

It is possible to suppose that the lower pregnancy rate in the cows fed on CS containing diets instead of BH may be related to a lower energy concentration of CS and the cows was in negative energy balance for long period than control. The results are in agreement with those obtained by Ben Salem and Bouraoui (2008) who stated that the reduction of negative energy balance of the cows allowing them to resume estrus earlier after parturition and therefore have better reproductive performance. The data are supported by results obtained by Ferguson *et al.* (1990), Sklan *et al.* (1991) and Ben Salem and Bouraoui (2008). Improvement of reproductive performance with CaSFO supplementation may be related to the increased proportion of EPA and DHA in the endometrial lipids and may inhibit secretion of uterine prostanoids and consequently decreased plasma progesterone which prevents the regression of the Corpus Luteum (CL) and thus improves embryos survival

(Sklan *et al.*, 1991; Mattos *et al.*, 2002). In this experiment it was observed that an improvement of reproductive performance was obtained in conjunction with higher milk production and that explain the importance of rumen protected fish oil supplementation to lessened the negative energy state and estrus cycles start and conception occur sooner (Staples *et al.*, 1998).

CONCLUSION

Results of this study suggest that addition of corn stalk up to 20% instead of berseem hay had no significant effect on milk production, improved milk-to-feed ratio and may be a good way to stabilize ruminal fermentation characteristics and reduce the risk of ruminal acidosis. Moreover calcium salt of fish oil supplementation at 11 g kg⁻¹ diet lessen the negative energy balance, improve DMI, milk production and reproductive performance of dairy cows.

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