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# Effect of bypass fat on milk lipid quality and reproductive efficiency of dairy cows

## Efecto de grasa sobrepasante sobre lípidos lácteos y eficiencia reproductiva en vacas lecheras

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### ARTICLE DATA

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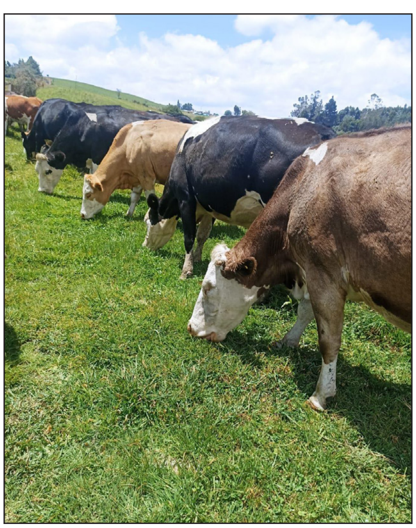

### ABSTRACT

Nariño (Colombia) has high dairy potential; however, their herds present difficulties in the nutritional component (energy imbalance) of the cows, affecting their productivity. This research evaluated the effect of a bypass fat supplement on lipid concentration in milk and the reproductive efficiency of dairy cows. The study was based on 21 Holstein x Simmental cows between first and third calving and the following was supplied: forage + concentrate (T1); forage + concentrate + 250 g/day of bypass fat (T2) and forage + concentrate + 250 g/day of bypass fat enriched with omega-three (T3). The experiment was conducted from day 15 pre-calving until day 105 of lactation. During this period, milk quantity, quality, composition, and reproductive behavior were recorded. For data analysis, a repeated measure mixed design with time-series data was used, where the fixed effects were the treatments, periods, and their interaction, the animals represented the random effect, and the covariable was the estimated milk yield during lactation. The results showed normal mobilization of adipose tissue; the compositional quality of the milk did not vary across treatments, although T2 presented a higher estimated production per lactation, and T3 presented a higher percentage of fatty acid C18:2. In the reproductive indicators, they were not influenced between the treatments; concluding that the base diet offered in the herd presents a nutritional balance appropriate to the requirements of the cows in production.

**Keywords**: bypass; quality; reproduction; omega 3 fatty acids; adipose tissue; ketosis.

### RESUMEN

Nariño (Colombia) tiene un gran potencial lechero; sin embargo, sus hatos presentan dificultades en el componente nutricional (desbalance energético) de las vacas, afectando su productividad. La presente investigación evaluó el efecto de un suplemento con grasa sobrepasante sobre la concentración de lípidos en la leche y la eficiencia reproductiva de las vacas. Para ello se utilizaron 21 vacas (Holstein x Simmental) entre segundo y tercer parto y se suministró: forraje + concentrado (T1); forraje + concentrado + 250 g/día de grasa sobrepasante (T2) y forraje + concentrado + 250 g/día de grasa sobrepasante enriquecida con omega tres (T3). El tiempo experimental fue desde el día 15 preparto hasta el día 105 de lactancia, donde se determinó la cantidad, calidad, composición de leche y el comportamiento reproductivo de cada animal. Para el análisis de los datos, se empleó un diseño mixto de medidas repetidas en el tiempo, donde los efectos fijos fueron los tratamientos, periodos y la interacción entre estos, mientras que el animal representó el efecto aleatorio, y la covariable fue la producción de leche estimada para la lactancia. Los resultados, revelaron movilización normal de tejido adiposo; la calidad composicional de la leche no varió entre los tratamientos, aunque T2 presento una mayor producción por lactancia estimada y T3 presento un mayor porcentaje del ácido graso C18:2. En los indicadores reproductivos no se vieron influenciados entre los tratamientos; concluyendo que, la dieta base ofertada en el hato, presenta un balance nutricional apropiados a los requerimientos de las vacas en producción.

**Palabras clave**: bypass; calidad; reproducción; ácidos grasos omega 3; tejido adiposo; cetosis.

## INTRODUCTION

The Colombian dairy industry has stood out in the last 30 years due to its dynamic nature, reflected by high growth in production. Colombia produces nearly 6717 million liters of milk annually and the growth in dairy production has reached almost 1.5% in recent years, parallel to the population growth (FEDEGAN, 2015). The specialized dairy industry in Colombia is distributed into three zones, known as dairy basins, including the Department of Nariño, the High Plains of Cundinamarca and Boyacá, and the Department of Antioquia in the northwest. These zones account for more than 70% of milk production in the dairy industry (Carulla & Ortega, 2016).

Forage sources for livestock feed in the high Tropics are commonly annual or perennial ryegrasses (*Lolium* sp.), natural or native forage mixed with naturalized grasses such as orchard grass (*Dactylis glomerata*), kikuyo (*Cenchrus clandestinum* Hochst. ex Chiov), a tufted grass (*Holcus lanatus*), alfalfa (*Medicago sativa*), canary grass (*Phalaris* sp.), and clover (*Trifolium repens*), as well as mixes among these (Solarte, 2009).

Galvis *et al*. (2005) mentioned that during the transition period (the period between three weeks before calving and three weeks after calving), there is a natural negative energy balance (NEB) given the high nutritional requirements resulting from the onset of milk production, while their contributions are low due to the limited intake of dry matter. This leads to delayed physiological reactivation of reproduction and loss of homeostasis, causing metabolic disorders and loss of milk volume and quality. Therefore, it is important to seek nutritional alternatives to help meet high energy demands in feasible, economical, and productive manners.

As an alternative to improve and supplement these energy deficits, cereal-based balanced feed is being used, mainly the commercial type, which leads to higher production costs. Given the high energy demand but low dry matter intake during the transition stage, certain technologies produce feed with high energy concentration, such as bypass fat (Campos *et al*., 2007).

Bypass fats were created as feed strategies to help overcome the energy deficit of high-yielding cows. This type of fat is inert in the rumen since it does not affect the cellulolytic activity of the bacteria while providing an important energy intake. Additionally, Hernández & Díaz (2011) indicated that bypass fat supplements elaborated with sources rich in polyunsaturated fatty acids can provide a nutraceutical effect in the final product through the incorporation of omega 3.

The success of a feed strategy in cattle relies on providing an adequate energy protein ratio to optimize nutrient use, so strategic supplementation must be adjusted to the animals' requirements (Sossa & Barahona, 2015). Milk yield largely depends on the reproductive performance of each female since the lactation cycle is restarted or renovated by gestation. The challenge of the dairy industry is to maintain high production levels without affecting reproductive parameters that depend on physiological, nutritional, genetic, and biological factors, as well as sanitation and management (Córdova & Pérez, 2005).

Given the above, this research evaluated the effect of incorporating bypass fat in the diet of high-yielding cows on the lipid concentration of milk and the reproductive activity of the cows. Furthermore, this study analyzed the metabolic behavior of the animals to understand essential factors in the specialized dairy systems that contribute to improving the competitiveness of dairy farmers and the economy of the livestock sector.

## MATERIALS AND METHODS

The experimental field stage was conducted between July and December 2016, under the animal experimentation license granted by the research ethics committee of Universidad Nacional de Colombia, located in Palmira.

**Location.** This research was conducted in the dairy basin of the municipality of Guachucal, Department of Nariño, geographically located at 0°54'52"N (borders Cuaspud) 1º01'53"N (borders Piedrancha and Sapuyes), and 77°35'57"W (Paja Blanca paramo) 77°48'55"W (Laguna de la Bolsa) West Longitude. The study herd is located at 3125 m asl., a zone with an average temperature of 10°C, relative humidity between 82-86%, and annual precipitation of 1017 mm. Further, this area corresponds to a Lower Montane Dry Forest (lm-DF) (Holdridge, 1971).

**Nutritional management of the animals.** The animals in the study were fed a grass-based diet composed of mixtures of ryegrass (*Lolium perenne*) and/or kikuyu (*Cenchrus clandestinus* Hochst. ex Chiov) see Table 1. Rotational grazing was implemented, in which the animals were fed *ad libitum*. The pastures for grazing had an initial margin of 10 $m^2$; then, an additional meter was assigned by moving the limits 20 to 25 times a day depending on the availability of forage in each paddock and food intake demands. At night, a permanent area of 30 $m^2$ was assigned. The rotation between pastures was done based on forage production and area, with an average occupation period of four to seven days per paddock. Additionally, the cows were supplemented with a commercial balanced feed, which was administered during the morning and afternoon milking. Specifically, 3 kg of prior to lactation concentrate supplementation for pre-calving cows (21 days before calving) and, for lactating cows, it was 1 kg of concentrate per 5 kg of milk produced. The bromatological analyses of the forage mix and commercial supplementations are described below.

Dry matter (DM) intake was determined according to the formula DMI=120/NDF and the amount of balanced feed intake was also calculated (Mertens, 2002). This provided an estimate of 14.95 kg of DM/cow/day; i.e., 2.67% of the live weight (LV) per animal, with an average weight of 560 kg.

**Table 1.** Nutritional composition of the forage mix

| VARIABLE | % |
|---|---|
| Dry matter | 22.9 |
| Ash | 8.87 |
| Protein | 17.66 |
| Ether extract | 2.42 |
| NDF | 60.61 |
| ADF | 33.86 |
| ADL | 9.4 |
| Hemicellulose | 26.75 |
| Cellulose | 24.46 |
| NFE | 10.44 |
| Energy (Kcal/100 g) | 430 |

Source: Laboratory of Animal Nutrition of Universidad Nacional de Colombia-located in Palmira.

**Experimental units and treatments.** For the selection of animals, criteria were taken into account, such as individual milk yield, calving date (i.e., at least 15 days before the start of the experiment), and that they were between second and third calvings. The work was conducted with 21 animals divided into three treatments, meaning seven Holstein x Simental cows, per treatment. The feed administered comprised forage + concentrate in the first treatment (T1), forage + concentrate + 250 g/day of bypass fat in the second treatment (T2), and forage + concentrate + 250 g/day of bypass fat enriched with omega 3 (2400 mg) in the third treatment (T3).

In the three experimental groups, 100 mL of milk samples were obtained and deposited in sterile plastic containers for the compositional analysis. Additionally, 500 mL samples of milk were extracted in dark glass jars for the fatty acid profile analysis; these samples were taken on days 45 and 90 for two samples per treatment. The samples were taken under biosafety parameters, using masks, gloves, and sterile containers, and they took place at the beginning of the morning milking, letting the selected cows enter first. All samples were transported to specialized laboratories at Universidad de Nariño in expandable polystyrene bags under cold chain conditions for adequate conservation.

Finally, data analysis involved repeated measures mixed design with time-series data. The fixed effects corresponded to the treatments, periods, and their interaction while the animals represented the random effect and the covariable was the estimated milk yield during lactation.

**Reproductive characterization.** The reproductive component was evaluated based on the following variables: number of services per conception (SC), days open (OD), and calving interval (CI). Data were obtained based on the information documented in physical notebooks that the farm had, to be later organized in an Excel database. The reproductive management and heat detection of each animal on the farm was done by the farm operator.

**Sample analysis.** The milk composition analysis was done by ultrasound using a Lactostar milk analyzer (Funke Gerber, Ekomilk, Berlin-Alm.)® to measure fat, protein, and total solids contents. The profile of the short, medium, and long-chain fatty acids and their main isomers were identified using a Shimadzu GC17A gas chromatography instrument using a DB-WAX column (Agilent Scientific, 30m x 0.25mm x 0.25μm), FID (Flamed Ionization Detection) at 280°C, split/splitless injection at 250°C in split mode 1:10, AP helium mobile phase at a flow of 1.0 mL/min.

**Statistical analysis.** A repeated measure mixed design with time-series data was used. The fixed effects were the treatments, periods, and their interaction, while the animal represented the random effect and the covariable was the estimated milk yield during lactation. The data were tested for normality and heteroscedasticity. The lowest values for the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to determine the best covariance structure. A comparison of means was used to determine statistically significant differences based on a DGC - Di Rienzo, Guzmán, & Casanoves test. Furthermore, a unidimensional descriptive and exploratory analysis was done based on average values and standard deviations. Data were processed using Statistical Analysis System (SAS) software®, version 9.0.

## RESULTS AND DISCUSSION

### Energy metabolism

**Effect of the diet on body condition.** The results did not show significant differences between treatments or sampling periods, indicating reduced mobilization of body reserves without excessive changes during the sampling period. These findings agree with reports by Aguilar *et al*. (2009b) and Tyagi *et al*. (2010) who did not find differences in body condition between animals with and without bypass fat supplementation. The animals showed reduced body condition after calving, which, according to Wathes *et al*. (2009), could be associated with a negative energy balance. Furthermore, Adrien *et al.*, (2012) established that an ideal body conditions score (BCS) for Holstein cows before calving should be between 3.0 and 3.5; additionally, the cows cannot lose more than one point in the BCS after calving until the production peak. Therefore, the absence of differences between the animals supplemented and not supplemented demonstrates that the feed base at the farm supplied the necessary energy so the animals did not have to mobilize body reserves during the evaluation period.

Hence, body condition is an important variable and should be evaluated in any experiment. Its evaluation should be done based on the initial state of the animals in each herd, and in this study, this variable is associated with the reproductive behavior of the herd.

**Milk yield and composition.** Table 2 describes the milk yield level and percentages of fat, protein, and total solids in milk for each treatment, in addition to the statistical significance between treatments, periods, treatment*period, and covariance. The analysis did not indicate significant differences between treatments for any of the variables. On the other hand, the evaluation between periods indicated highly significant differences in milk yield. The highest yield was found at day 45 of lactation, which differed from the other periods. Furthermore, there were significant differences in the percentage of total solids, observing the highest concentration at day 15 of lactation. Moreover, the interaction between treatment and period showed differences in fat and total solids contents.

**Milk yield.** Milk yield is affected by several factors associated with the animal and the environment (Cañas *et al.*, 2012); nevertheless, this research did not show significant differences between treatments. These results agree with Crespi *et al*. (2014), Prieto *et al*. (2016), and Duque *et al*. (2013), who reported that milk yield and composition were not influenced by supplementation with any type of fat source. When yield was adjusted to the end of the lactation period (305 days), treatments T2 and T3 with supplementation showed higher values than T1 without supplementation, possibly due to improved energy efficiency and use.

Moreover, there were highly significant differences in milk yield between periods, indicated by the highest production at day 45 of lactation (period 4). Calvache & Navas (2012) state that the number of calvings, cow age, and genetic composition, among others, can affect milk yield. On the other hand, during the lactation peak, the main factor is the time of calving, which largely determines the availability and quality of the feed administered to the animals. These results agree with the findings of this research since the animals were permanently fed, including the feed base (forage) and supplementation (concentrate). Likewise, it is important to mention that the Holstein breed reaches its peak of production at 45 days post-calving, as pointed out by Cañas *et al*. (2012), reporting that for Holstein cattle in Colombia, the highest milk production occurs around day 44.

**Fat, protein, and total solids in milk.** This study did not determine significant differences in fat, protein, and total solids contents between treatments. This can be explained by Crespi *et al*. (2014), who mentioned that, if the complete diet does not contain more than 6% of ether extract, there would be no expected differences in the fat levels of milk. Regarding protein content, the results agree with reports by Prieto *et al*. (2016), who did not find significant differences in milk yield and fat and protein contents between treatments. These findings indicate that the diet administered supplies the rumen with sufficient nutrients for normal ruminal fermentation; furthermore, the addition of protected fats did not affect milk synthesis. The fat and protein contents did not show differences between sampling periods. Conversely, the percentage of total solids showed differences, observing the highest value at day 15 of lactation, which can be attributed to the onset of milk production and the synthesis of milk nutrients.

**Table 2.** Variables associated with milk yield and composition and their statistical significance.

| Variable | T1 | T2 | T3 | Statistical effect | | | |
|---|---|---|---|---|---|---|---|
| | | | | treatment | period | tt*per | covariance |
| Milk yield (L/d) | 21.8 | 23.7 | 22.4 | 0.0892 | 0.009 | 0.704 | <0.0001 |
| Fat (%) | 3.8 | 3.7 | 3.6 | 0.3849 | 0.1756 | 0.0209 | *ns |
| Protein (%) | 3.18 | 3.12 | 3.18 | 0.4653 | 0.0892 | 0.1432 | *ns |
| Total solids (%) | 12.46 | 12.4 | 12.2 | 0.4907 | 0.0439 | 0.0172 | *ns |

Significant differences were established at $p<0.05$. *ns indicates that the covariance was not significant in the first statistical test; therefore, it was removed from the model for this trait (David, 2018).

**Fatty acids profile.** The results of the two sampling periods (days 45 and 90 of lactation) for each treatment are shown in Table 3. In T1, there are high contents of saturated, monounsaturated, and polyunsaturated fatty acids for the first sampling period (45 days), while the second period (90 days) showed an increase in saturated fatty acids, a reduction in monounsaturated fatty acids, and an absence of polyunsaturated fatty acids. These findings could be due to the type of feed base (ryegrass). According to Aguilar *et al*. (2009a), kikuyo-based diets show a higher concentration of polyunsaturated fatty acids and conjugated linoleic acid (CLA) (cis 9, trans 11) in milk fat compared to ryegrass-based diets. Furthermore, the authors mention that the lowest fat production, specifically, long-chain fatty acids, is due to a lower fiber intake and, consequently, reduced ruminal pH. This leads to a lower fat percentage and a decrease in isomerization and biohydrogenation.

**Table 3.** Fatty acids profile of the fat component of milk

| | | **45 days of lactation** | | | |
|---|---|---|---|---|---|
| **Fatty acid** | **Nomenclature** | **T1** | **T2** | **T3** | **p-value** |
| Isobutyric | | 0.50 ± 0.04a | 0.5 ± 0.04a | 1 ± 0.04b | <0.0001 |
| Butyric | C4:0 | 0.30 ± 0.04a | 2.30 ± 0.04b | 1.70 ± 0.04c | <0.0001 |
| Caproic | C6:0 | - | 0.5 ± 0.02b | - | <0.0001 |
| Caprylic | C8:0 | 0.50 ± 0.04a | 0.90 ± 0.04b | 2.40 ± 0.04c | <0.0001 |
| Capric | C10:0 | 3.5 ± 0.03a | 0.20 ± 0.03b | 0.70 ± 0.03c | <0.0001 |
| Lauric | C12:0 | 3.80 ± 0.02a | 3.90 ± 0.02b | 3.5 ± 0.02c | <0.0001 |
| Myristic | C14:0 | 16.7 ± 0.02a | 19.2 ± 0.02b | 15.3 ± 0.02c | <0.0001 |
| Pentadecanoic | C15:0 | - | - | - | |
| Palmitic | C16:0 | 39.1 ± 0.02a | 42.1 ± 0.02b | 34.5 ± 0.02c | <0.0001 |
| Stearic | C18:0 | 9.2 ± 0.02a | 14.9 ± 0.02b | 15.10 ± 0.02c | <0.0001 |
| Oleic | C18:1 | 23.6 ± 0.02a | 15.2 ±0.02b | 25.9 ± 0.02c | <0.0001 |
| Linoleic | C18:2 | 2.7 ± 0.01b | - | - | <0.0001 |
| | | **90 days of lactation** | | | |
| **Fatty acid** | **Nomenclature** | **T1** | **T2** | **T3** | **p-value** |
| Isobutyric | | 1 ± 0.05ª | 0.80 ± 0.05b | 1 ± 0.05a | 0.0103 |
| Butyric | C4:0 | 1.70 ± 0.04a | 1.10 ± 0.04b | 1.70 ± 0.04a | <0.0001 |
| Caproic | C6:0 | 1.60 ± 0.03a | 1.10 ± 0.03b | 1.10 ± 0.03b | <0.0001 |
| Caprylic | C8:0 | 1.70 ± 0.03a | 1.10 ± 0.03b | 1.10 ± 0.03b | <0.0001 |
| Capric | C10:0 | 3 ± 0.03a | 2.3 ± 0.03b | 1.8 ± 0.03c | <0.0001 |
| Lauric | C12:0 | 4 ± 0.03a | 3.3 ± 0.03b | 3 ± 0.03c | <0.0001 |
| Myristic | C14:0 | 14 ± 0.02a | 12.5 ± 0.02b | 12.2 ± 0.02c | <0.0001 |
| Pentadecanoic | C15:0 | 2 ± 0.13a | 1.6 ± 0.13a | 1.7 ± 0.13a | 0.1037 |
| Palmitic | C16:0 | 40 ± 0.18a | 39.2 ± 0.18b | 39 ± 0.18b | 0.0027 |
| Stearic | C18:0 | 9 ± 0.13a | 9.5 ± 0.13b | 9.3 ± 0.13ab | 0.0443 |
| Oleic | C18:1 | 22 ± 0.13a | 26.2 ± 0.13b | 27.3 ± 0.13c | <0.0001 |
| Linoleic | C18:2 | - | 1.2 ± 0.13a | 2 ± 0.13b | <0.0001 |

Statistically significant differences were established at p<0.05. Different letters indicate differences between treatments. (-) indicates the absence of fatty acids for a given treatment. Source (David, 2017).

Moreover, T2 showed a lower percentage of saturated fatty acids in the second period (90 days), while the percentages of mono and polyunsaturated fatty acids increased. García *et al*. (2014) mention that 50% of pre-formed fatty acids derived from intestinal triglycerides or non-esterified fatty acids (NEFA) mobilized from the adipose tissue, such as palmitic and stearic acids, are transferred to milk. In turn, these fatty acids are capable of reducing the de novo synthesis of short-chain fatty acids used for fat synthesis in the mammary gland.

Finally, in T3, with the addition of omega 3, a lower percentage of saturated fatty acids was observed at 90 days of lactation, while the percentage of monounsaturated and polyunsaturated fatty acids increased.

The results can be explained by Moioli *et al*. (2007), who found that high amounts of trans stearic acid inhibit de novo synthesis and activity of the Δ9-stearoyl-CoA desaturase, limiting the conversion of saturated fatty acids and increasing the amount of mono and polyunsaturated fatty acids (C18:2). Similarly, Glasser *et al*. (2013) mention that greater availability of C18 fatty acids, resulting from a larger feed supply, leads to a reduced concentration of medium-chain fatty acids. Furthermore, the secretion of C18:0 in milk can be promoted by the administration of C18:0 in the feed or the supply of unsaturated fatty acids due to total or partial hydrogenation in the rumen. This study suggests that long-chain fatty acids were incorporated mainly through the forage diet since the bypass fat administered was inert in the rumen and large mobilization of adipose tissue was not observed. Furthermore, linoleic acid (C18:2) was detected in the milk, indicating that the addition of omega 3 to the diet results in milk with improved traits, including nutraceuticals.

**Reproductive activity.** The reproductive parameters evaluated in this study were calving interval (CI), services per conception (SC), and days open (OD). No significant differences were found between treatments ($p>0.05$) for any of the traits. The average CIs were 363, 357, and 375 days and the average DO was 85, 79, and 90 days for treatments 1, 2, and 3, respectively. Furthermore, the average SC were 1.6 for T1 and T3 and 1.5 for T2.

According to Tyagi *et al*. (2010), bypass fat supplementation in high-yielding mixed breed cows reduces the time required to restart the estrous cycle and the calving to the first service interval. Similarly, Duarte *et al*. (2016) reported lower calving intervals in cows supplemented with bypass fat. The same was not observed in this study possibly since only 1.53% of bypass fat was used. The recommended amount of bypass fat is 280 g to achieve better results; however, this research used a lower amount due to the commercial cost of the product. Despite this, the best results were found in T2 with bypass fat supplementation.

## CONCLUSIONS

This research demonstrates that supplementation with bypass fat affected milk yield and nutritional composition in T2: forage + concentrate + 250 g/day of bypass fat and in T3: forage + concentrate + 250g/day of bypass fat enriched with omega 3 (2400mg). Furthermore, the addition of omega 3 improved the fatty acids profile in milk, demonstrating a greater presence of C18:2 fatty acids, which confer nutraceutical properties.

Regarding reproductive activity, there were no statistical differences between the treatments or the variables evaluated, concluding that the supplement and the amount provided had no nutritional influence on the reproductive activity of dairy cows.

**Conflict of interests**: The authors declare that there are no conflicts of interest.

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