

Effect of three levels of the inclusion of excess fat on the ovarian cyclicity of dairy herds in the Ecuadorian Andes

Efecto de tres niveles de la inclusión de grasa de sobrepaso sobre la ciclicidad ovárica de hatos lecheros en los Andes Ecuatorianos

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ABSTRACT

This study focuses on cyclicity differently since it assesses the effect of fat on the times and structures of ovarian activity, which are variables closely related to the reproductive efficiency of herds typical of the southern region of the Ecuadorian Andean region. The use of "by pass" fat has positive effects on the ovarian activity and presence of ovarian structures of the animals, even though the animals are not highly productive. This study focused on evaluating the effect of three levels of "by pass" (GB) fat diets: GB1% (154-175 gr), GB2% (296-361gr) GB3% (475-538 gr) and a control (GB0%); in a group of 60 Holstein dairy cows, clinically healthy, from 2 to 5 births and with body condition from 3 to 3.5. The size and number of ovaries were analyzed as a reference for ovarian activity; and the presence of follicles and corpora lutea as ovarian structures. Significance tests were performed to determine the differences of the variables, concluding that the addition of "by pass" fat advances the presence of ovarian activity, and influences the estrous cycles. The positive effect of the same on body condition and production was also confirmed.

Keywords: by pass fat, ovarian structures, ovarian activity, typical herds.

RESUMEN

Este estudio se enfoca de forma diferente sobre la ciclicidad ya que evalúa el efecto de la grasa en los tiempos y estructuras de la actividad ovárica, que resultan variables estrechamente relacionadas a la eficiencia reproductiva de los hatos típicos de la región austral de región andina ecuatoriana. El uso de grasa "by pass" tiene efectos positivos en la actividad ovárica y presencia de estructuras ováricas de los animales, a pesar de que los animales no sean altamente productores. Este estudio se enfocó en evaluar el efecto de tres niveles de dietas de Grasa "by pass" (GB): GB1% (154-175 gr), GB2% (296-361gr) GB3 % (475-538 gr) y un control (GB0%); en un grupo de 60 vacas lecheras Holstein, clínicamente sanas, de 2 a 5 partos y con condición corporal de 3 a 3.5. Se analizó el tamaño y el número de ovarios como referencia de la actividad ovárica; y la presencia de folículos y cuerpos lúteos como estructuras ováricas. Se realizaron pruebas de significancia para determinar las diferencias de las variables concluyendo que la adición de grasa "by pass" adelanta la presencia de actividad ovárica, e influye en los ciclos estrales. Se confirmó también el efecto positivo de la misma sobre la condición corporal y producción.

Palabras claves: estructuras ováricas, actividad ovárica, grasa "by pass", hatos típicos.

INTRODUCTION

The long periods between births affect the profitability of the herds, caused by prolonged anovulatory anestrus states ([Rhodes et al., 2003](#)), there being a high difficulty to reach a restart of the ovarian activity ([Báez et al., 2009](#)). For this to happen, basically two events must occur: the involution of the uterus and the reactivation of the hypothalamic-pituitary-ovary axis ([Morales et al., 2012](#)), associated with the culmination phase of uterine involution ([Palomares, 2008](#)). The calving-first ovulation interval in dairy cattle is of 43-45 days ([Becerra et al., 2008](#)), being able to reach even around 85 days due to a late uterine involution.

The gonadotropin concentrations are very low at the end of pregnancy ([Robson, et al., 2008](#)). The development of follicular waves occurs early ([Kawashima et al., 2011](#)) after childbirth, there being an increase in FSH ([De Nava et al., 2012](#); [Báez et al., 2009](#); [Rivas et al., 2011](#)) from 5 to 10 days; while the pulsatility of LH is detected around 10 days ([Rhodes et al., 2003](#)). A dominant follicle begins to grow 10 to 14 days postpartum ([Wiltbank et al., 2002](#)) ([Silva et al., 2011](#)), causing the first postpartum ovulation, resulting from the premature release of prostaglandin F2a by the uterus and the interaction of the corpus luteum ([Becerra et al., 2008](#)) ([Motta et al., 2011](#)).

The main influential factors in postpartum anestrus are: nutrition (energy balance and body condition), age at childbirth, seasonality and peripartum diseases ([Rhodes et al., 2003](#), [Morales et al., 2012](#), [Becerra et al., 2008](#); [Silva et al., 2011](#)); causing the different types of anestrus ([Rhodes et al., 2003](#); [Morales et al., 2012](#); [Wiltbank et al., 2002](#)).

Restricted energy consumption affects postpartum ovarian activity ([Espinoza et al., 2010](#)), causing a progressive loss of body condition (C.C.) ([Giraldo et al., 2012](#)); although it is not possible to accurately measure the energy balance ([Rhodes et al., 2003](#); [Montaño et al., 2005](#); [Hernández and Díaz, 2011](#)). Cyclic reproductive indicators can be a tool to quantify this phenomenon.

Lipid supplementation is positively associated ([Giraldo and Uribe 2012](#)); however, the level of fat supplementation "by pass" is variable, and should not exceed 4% of the ration ([Herrera et al., 2012](#), [Tyagi et al., 2010](#), [Salas et al., 2011](#)). This supplementation increases the energy density of the diet, improving the productive and reproductive behavior ([Giraldo and Uribe, 2012](#), [Hernández and Díaz 2011](#), [Herrera et al., 2012](#), [Tinoco and Orozco 2012](#)).

Fats "by pass" consist of a source of unsaturated fatty acids, the most important of linolenic and linoleic fatty acids ([Duque et al., 2011](#)), and to be protected are not used by rumen microorganisms ([Duque et al., 2011](#)). The circulating free fatty acids (NEFA) are metabolized by three routes: oxidized by the liver and skeletal muscle; they experience β -oxidation and undergo a re-esterification to triglycerides in the liver; or they are used by the mammary gland ([Rivas et al., 2011](#); [Bobe et al., 2004](#)). The formation of ketone bodies is directly

proportional to the oxidation of fatty acids ([Zeoula and Ferreira, 2001](#)); while energy restriction has no effect on lipolysis in early lactation ([Wadhwa, et al., 2012](#)).

It is stated that the greatest and best effects in the utilization of "by pass" fats are achieved in the first third of lactation, related to a better use of energy and not with an increase in the supply of energy ([Giraldo and Uribe 2012](#)), exist simultaneously effects on the hypothalamic-pituitary-ovarian axis ([Staples et al. 1998](#)), hormone secretion, ovarian activity ([Herrera et al., 2011](#)), voluntary consumption ([Garcia 2012](#)) and production ([Martinez et al., 2010](#)).

Saturation of polyunsaturated fatty acids (PUFA) in the rumen occurs as defense mechanisms of ruminal microorganisms, decreasing their toxicity for ruminal microbiota ([Angel 2009](#)). When a source of active fat (not surpassing) with high levels of PUFA is used in the diet of ruminants, most of these elements are lost by the action of bio-hydrogenation, limiting their use ([Hernández and Díaz 2011](#); [Salvador et al. 2011](#)).

The present study evaluated the effect of the addition of different percentages of fat "by pass", as energy supplement in ovarian activity and return to the cyclicity of mestizo dairy cows from a typical property of the Victoria del Portete parish (Cuenca).

MATERIAL AND METHODS

The present investigative work was carried out in the Hacienda "La Rosa" located at 2850 masl, 60 adult bovine females from 2 to 5 births were used, with body condition at birth from 3 to 3.5, without apparent reproductive or metabolic problems at birth; under a system of grazing on 30 hectares of a forage mix of *Lolium perenne*, *Dactylis glomerata*, *Trifolium repens*, *Trifolium pratense*; *Pennisetum clandestinum* and *Holcus Lanatus* (ash: 10%, ether extract: 2.82%, protein: 16.36% and fiber: 22.16%, with a calculated energy requirement for cows with an estimated weight of 600 kg, net energy of lactation and maintenance 19.4 Mcal, expenditure in mobilization 4.02 Mcal and expense in regulating the temperature 5.4 Mcal. The animals were fed with "by pass" fat: Toco BP® (La Fabril SA) mixed in 2 kg/cow/day of Super-milk Proganized concentrate ® (protein: 14%, fat: 3%, source: Pronaca) and supplemented with mineral salt (Ca 20% and P10%).

The amount of "by pass" fat that was supplied was calculated according to the treatments proposed and according to the weight interval, prior to obtaining the average and standard deviation of the group of animals involved in the experiment, [table 1](#). It was established a period of habituation to the fat "by pass" (5 days before) and food was kept in the animals until day 60 postpartum.

Treatments were divided into doses in different percentages (1, 2 and 3 %) of bypass fat, supplied twice a day. The dependent variables were: ovarian activity (measured at intervals of 10 days from delivery until 60 days postpartum), presence of follicles, corpora lutea, total

number of follicles found per animal and follicular size (transrectal ultrasound "Sonoescape A6"), production (liters per day) and body condition (scale 1 to 5).

The statistical package INFOSTAT (2011) was used to determine the differences between treatments; they were compared with a Chi square test and descriptive statistics were used to represent these differences.

RESULTS

The follicular behavior of the animals was analyzed according to the ovarian activity and the presence of ovarian structures present during the study, where there is influence of the fat "by pass" in the cyclic activity of the animals ($P < 0.05$) and on the different types of ovarian structures ($P < 0.05$), [table 2](#).

Reproductive Variables

Ovarian activity increases from day 30 postpartum, due to the increase of ovarian structures, summarized in [Table 3](#); where there are no statistical differences in the cyclic activity ($P > 0.05$), due to the number of days; while in the effect of "by pass" fat, the difference is significant. Regarding the presence or absence of ovarian structures ($P < 0.01$), the ovarian activity and presence of ovarian structures is significantly higher ($P < 0.01$), according to the location of the uterine horn.

Ovarian activity was defined by the presence or absence of corpora lutea and follicles as indicators of ovarian activity. In this case, both the treatment GB0% and GB3% show a greater percentage of absence of ovarian structures in proportion, this difference being more marked in the right horn. The presence of follicles (ovarian structures) is greater in the GB1% and GB2% treatments. These values hold significance in the right horn on the left, demonstrating greater ovarian activity in this area. There are at the same time a greater number of corpora lutea in the treatment GB1%.

When comparing the effect of days in the ovarian cycle, it can be evidenced the increase of the same after 30 days, highlighting the treatments GB1% and GB2%. In the percentage of animals that present follicular activity, the control treatment (GB0%) presents lower values of

Table 1. Net Breastfeeding Energy (ENL) supplied to each group of cows by means of fat supply "by pass"

Groups/# animals	Range	Grams	Quantity of ENL	Unit
3%(15)	Minimum	538	2.37	Mega calories
	Maximum	475	2.10	
2%(15)	Minimum	361	1.59	
	Maximum	296	1.31	
1%(15)	Minimum	175	0.77	
	Maximum	154	0.68	
0%(15)	-	0	0.00	

corpus luteum presence (37%^a) in the accumulated percentages ($P < 0.05$), compared to the GB1% treatments (50%^b); GB2% (43%^b) and GB3% (56%^b) respectively.

Table 2. Percentages of animals that showed ovarian activity.

	Days	Left Ovary		Right Ovary	
		C.L.	Follicle	C.L.	Follicle
GB0%	10	6.7%	93.3%	0.0%	80.0%
	20	0.0%	80.0%	0.0%	100.0%
	30	6.7%	86.7%	13.3%	60.0%
	40	6.7%	86.7%	6.7%	80.0%
	50	20.0%	66.7%	0.0%	86.7%
	60	13.3%	86.7%	0.0%	73.3%
	X ⁻	8.9%	83.3%	3.3%	80.0%
GB1%	10	13.3%	86.7%	0.0%	100.0%
	20	0.0%	93.3%	0.0%	93.3%
	30	26.7%	73.3%	0.0%	100.0%
	40	33.3%	66.7%	0.0%	100.0%
	50	6.7%	93.3%	0.0%	100.0%
	60	6.7%	93.3%	13.3%	86.7%
	X ⁻	14.4%	84.4%	2.2%	96.7%
GB2%	10	0.0%	100.0%	0.0%	100.0%
	20	0.0%	100.0%	0.0%	100.0%
	30	60.0%	40.0%	6.7%	93.3%
	40	13.3%	86.7%	6.7%	73.3%
	50	0.0%	93.3%	0.0%	80.0%
	60	0.0%	100.0%	0.0%	100.0%
	X ⁻	12.2%	86.7%	2.2%	91.1%
GB3%	10	6.7%	93.3%	0.0%	93.3%
	20	0.0%	86.7%	13.3%	80.0%
	30	13.3%	73.3%	6.7%	80.0%
	40	0.0%	93.3%	6.7%	53.3%
	50	40.0%	60.0%	0.0%	86.7%
	60	20.0%	73.3%	6.7%	53.3%
	X ⁻	13.3%	80.0%	5.6%	74.4%

Tables 3. Animals with cyclic activity in relation to the Corpus Luteum.

Days	GB0%	GB1%	GB2%	GB3%
10	3%	7%	0.0%	3%
20	0.0%	0.0%	0.0%	7%
30	10%	10%	33%	10%
40	10%	17%	7%	3%
50	10%	3%	10%	20%
60	7%	10%	7%	13%
X ⁻	6.6%	7.8%	9.5%	9.3%

The treatments do not affect ($P > 0.05$) the number of follicles, where the maximum value was 6.37 (GB2%) and the minimum of 5.61 (GB1%). The size of the follicles did not show statistical differences either ($P > 0.05$), where the maximum value was 7.43 mm (GB%2) and 6.68 mm (GB%3). Regarding the number of follicles and their size, differences were found ($P < 0.05$) between the different days of the study. The number of follicles does not have statistical differences ($P < 0.05$) in relation to the left (5.39^a) and right (6.8^a). With regard to multifactorial analyzes, it can be affirmed that postpartum days affect ($p < 0.05$) in general all treatments.

It was also proceeded to analyze the correlation of cyclicity variables ($P < 0.05^*$ and $P < 0.01^{**}$) with each other; where the larger the number, the lower the number of follicles (-0.39^{**}); at the same time as the postpartum days pass, the number of follicles tends to decrease (-0.19^{**}), while the size increases (0.09^*).

Body and productive condition variables

The body condition (C.C) is affected by the fat "by pass" where the control (GB0%) had an average of 2.60^a; while the treatments had an average of 2.97^b (GB% 1), 2.98^b (GB% 2) and 2.99^b (GB% 3) respectively; values that support what has already been cited by [Roche et al., \(2009\)](#) and supported by a meta-analysis conducted by [Rodney et al. \(2015\)](#); maintaining a positive relationship (0.10^*) with postpartum days.

Finally, the influence of by-pass fat on production was analyzed, where the inclusion of this influence significantly influences production, [table 4](#).

DISCUSSION

The data are similar to the results obtained by [Tyagi et al. \(2010\)](#) and those of [Staples et al. \(1998\)](#), who determined in several studies that there is a tendency to improve conception rates with the use of fat (calcium soaps, fish oil and tallow). By increasing the availability of fatty acids to be absorbed in the intestine of ruminants, these acids can more easily reach the reproductive tissues and improve fertility ([Ortega et al., 2012](#)).

In this investigation the statistical effect of the fat "by pass" in the increase of the number of follicles of the treatments could not be evidenced with averages that go from 6.23^a to 6.37^a, and averages of the control of 5.61^a; these values being significantly lower than those of said study, which is related to different management and genetic conditions of this study. [Takahashi et al., \(2013\)](#), on the other hand, obtained a greater number of follicles ($14.9^c \pm$

Table 4. Effect of by-pass fat on production (P = 0.05)

TREATMENT	AVERAGE PRODUCTION	%CV
GBP0%	15.16 ^a	8.4%
GBP1%	18.15 ^b	8.1%
GBP2%	18.31 ^b	7.4%
GBP3%	19.11 ^b	10.1%

8.9) compared to control ($10.0^b \pm 7.6$), taking into account that the study mentioned also focused on other factors.

Statistical analysis of ovarian activity (presence of ovarian structures) in this study obtained significant differences for the location (horn), where there is greater activity. The animals treated with "by pass" fat (GB1% and GB2%) showed a higher specific activity in this location than in the control group (GB0% = 7.8%) and the treatment with the highest percentage (GB3% = 6.7%); confirming that very high diets can alter ovarian cyclicity, [Duque et al. \(2013\)](#), as well as the low ones [Ramteke, et al. \(2014\)](#).

The results in favor of diets at 1% of fat supplementation based on the intake of dry matter, is due to the fact that the lipids offered are protected, reducing the impact of the action of the rumen ([Hernández and Díaz 2011](#); [Rhodes et al., 2003](#); [García, 2012](#)).

[Beam et al. \(1997\)](#), determined that the rations of high energy content increase the diameter of the follicles. The size of the follicles in this study was not significant between treatments, with a maximum value (7.41mm) of GB2%; values that are numerically superior to the others studied.

[Beam et al. \(1997\)](#), indicate that up to 42 days postpartum in high production cows; while in this study the peak of ovulation quantified by follicular activity is achieved after 30 days, obtaining statistically higher values in the intermediate treatments (GB1% and GB2%).

To improve the reproductive function of dairy cows through lipid supplementation, it is necessary to supply lipids containing essential fatty acids (polyunsaturated fatty acids or PUFAs: oleic, linoleic, linolenic) and be protected so as not to be affected by ruminal action, or alter the degradation of cellulose present in forages ([Hernández and Díaz 2011](#)).

When the energy requirement is greater than its supply, the lipolysis rate is higher than lipogenesis, so the cow loses body condition and increases the serum levels of non-esterified fatty acids (NEFA) ([Roche et al., 2009](#)) . The changes of C.C. of this study confirm the action of "by pass" fat where the intake of metabolizable energy increases energy reserves and body condition ([Hills et al., 2015](#)).

CONCLUSION

When used in recommended doses, the use of "by pass" fat has positive effects on ovarian activity (time), and the presence of ovarian structures (number) of the animals, even though the animals are not highly productive; therefore, intermediate by-pass fat treatments (GB% 1 and GB% 2) obtain better reproductive results than the control and GB% 3 groups.

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