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DYNAMICS OF THE LARGEST OVARIAN FOLLICLE DURING PUERPERIUM IN DAIRY COWS FED WITH DIFFERENT TYPES OF UREA AS A PARTIAL SUBSTITUTE FOR SOYBEAN MEAL)

(*Dinâmica do maior folículo ovariano no puerpério em vacas leiteiras alimentadas com diferentes tipos de ureia como substituto parcial do farelo de soja*)

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ABSTRACT - This study evaluated the effects of partial substitution of soybean meal with conventional or slow-release urea on ovarian follicular dynamics during puerperium in Holstein cows. Forty-four recently calved cows were assigned to one of three diet groups: soybean meal group (SG; n = 12; 900 g/day/cow); conventional urea group (CUG; n = 16; 130 g/day); or slow-release urea group (SUG; n = 16; 147 g/day). Each cow received the diet daily for 10 days before their expected calving day, and for up to 39 days (d39) after calving. Ultrasound examinations were conducted at 2 to 3-day intervals, between d6 and d39 of puerperium. Data on the growth of the largest follicle during this time were analyzed using SAS. There was no significant difference in the diameter of the largest follicles observed in the three groups between d6 and d21, On d23 group SUG significantly differed (P < 0.05) from the other two groups

Keuwords - follicular dynamics; livestock conventional urea; ovarian ultrasonography; puerperium; slow-release urea.

RESUMO - O estudo avaliou os efeitos da substituição parcial do farelo de soja por ureia pecuária convencional ou ureia de liberação lenta sobre o desenvolvimento do maior foliculo ovariano durante o puerpério em vacas da raça Holandesa. Quarenta e quatro vacas recém-paridas foram divididas em três grupos: grupo farelo de soja (GS; n=12; 900g/dia/vaca); grupo ureia convencional (GUC; n=16; 130g/dia/vaca); ou grupo ureia de liberação lenta (GUL; n=16; 147g/dia/vaca). Cada vaca recebeu a dieta diariamente a partir do dia 10 antes da data esperada para o parto até 39 dias (d39) após o parto. Exames de ultrassonografia foram realizados em intervalos de 2 ou 3 dias, entre d6 e d39 do puerpério. Dados do crescimento do maior folículo encontrado durante este período foram analizados utilizando o SAS. Não houve diferença significativa no diâmetro dos maiores folículos observados nos três grupos entre d6 e d21. Em d23, o grupo GUL diferiu significativamente (P < 0,05) dos outros dois grupos. Não foi observada diferença no diâmetro dos folículos dos diferentes grupos a partir de d25 até o final do experimento. Conclusão: a substituição parcial do farelo de soja com ureia pecuária convencional ou de liberação lenta pode ser feita para vacas leiteiras no puerpério sem consequências negativas na atividade folicular ovariana.

Palavras-chave - dinâmica folicular; puerpério; ultrassonografia ovariana; ureia de liberação lenta; ureia pecuária convencional.

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INTRODUCTION

Substitution of foods high in protein (e.g., soybean meal) with lower cost alternatives (e.g., urea) has become a common practice in livestock production. Microbial protein of high biological quality is produced from non-protein nitrogen (NPN) sources in ruminants (Lapierre and Lobley, 2010; NRC, 2001). The release of ammoniacal nitrogen (N-NH3) by ureases from the ruminal microbiota may absorb this surplus and promote changes in urea nitrogen levels in blood and urine[1]. Therefore, insufficient urea in the diets of dairy cows may increase the urea nitrogen potential in milk to above ideal levels (Imaizumi *et al*., 2010).

Dawuda *et al.* (2002) reported a reduction in the rate of embryo production when urea was fed to cows during the growth of ovarian follicles. This suggests a potentially negative effect of urea on follicle quality. However, these authors also concluded that cows can adapt to changes in their diet within 10 days. Grant *et al.* (2013) reported that diets that produced significantly higher levels of urea nitrogen in blood and milk did not cause significant changes in uterine pH, suggesting that other mechanisms might be involved in reducing fertility in cows.

There are several kinds of urea that can be fed to dairy cows. Several references indicate good commercial feeds for substitution of protein foods with slow-release urea (Laven *et al*., 2004; Chedthong and Wanapat, 2010). Souza *et al*. (2010) concluded that the partial substitution of soybean meal with slow-release urea can be given without damage to productivity in high performance dairy cows. Partial substitution of soybean meal with slow-release urea also produces satisfactory results when the milk is used to make cheese (Souza *et al*, 2015). Aguiar *et al*. (2013) also reported that substitution of up to 1.75% urea from dry matter in the diet maintained identical cheese quality.

Although there is evidence for satisfactory productivity from the use of urea, there are few studies on the effect of urea on ovarian follicular activity (follicular dynamics) in bovines. Technically, the slow-release protected urea is "shielded" by protein nanoparticles, and so is resistant to thermal processing. This provides an advantage over other similar products that may, for example, undergo changes in their protection during the pelletizing process. This technique has shown promise in reducing ruminal N-NH3 and increasing the ruminal microbiota population compared to conventional urea (Cherdthong and Wanapat, 2010).

Previous studies detected a high correlation between serum urea levels in blood plasma and ovarian follicular fluid during puerperium in high milk production cows (Leroy *et al*. 2004). Hammon *et al*. (2005) reported that diets favoring high levels of urea nitrogen in the plasma reduced fertility in dairy cows during puerperium, since they affected the quality of the preovulatory follicle during estrus and the uterine environment during the luteal phase of the estrous cycle.

This study tested the hypothesis that the addition of slow-release urea to the diet of dairy cows during puerperium does not alter follicular recruitment, selection, or the dominant follicle (DF) in follicular dynamics, or compromise other aspects of reproductive activity in high production cows. As such, this study verified the effects of substituting soybean meal (control) for conventional or slow-release urea on ovarian follicular dynamics during puerperium in high production Holstein cows.

MATERIAL AND METHODS

Study site and animals

The study was conducted at the Experimental Farm of Pontifical Catholic University of Paraná, located at 25° 39′ 28″ S, 49° 18′ 28″ W. The farm was equipped with a *free-stall* system for dairy cows (Black and White Holstein breeds), and average milk production was 35 liters/day (range 20-60). The cows were milked twice a day in a tandem rotating milking parlor (carousel), with the capacity for milking 12 cows simultaneously and collecting data via Dairy Plan® software. Forty-four cows, all multiparous, and having a body condition score (BCS) between 3.5 and 4.75 (1 = thin, 5 = obese [Ferguson *et al*. 1994]), normal calving, and without reproductive disorders, were assigned to one of three groups during early puerperium. Animals were assigned with respect to the order of lactation, age, milk production, and lactation days, with as homogeneous a distribution among the groups as possible.

Food Management

The standard diet of the animals was based on spring-summer (*Cynodon dactylon*) and winter (*Lolium multiflorum*) forage, with corn silage, mineral salt, and water offered *ad libitum.*

The cows were assigned to one of three groups, and given one of three different diets during the experimental period (puerperium):

1. Soybean meal Group (control) (SG; n = 12): daily diet based on corn silage containing 900 g of soybean meal without addition of urea (twice a day; morning and afternoon)
2. Conventional livestock urea group (CUG; n = 16): daily diet based on corn silage with soybean meal substituted with conventional urea (130 g) and corn meal (800 g) (twice a day)
3. Slow-release urea group (Sion Nanotech, São Paulo, Brazil) (SUG; n = 16): daily diet based on corn silage with soybean meal substituted with slow-release protected urea (147 g; manufacturer recommendations) and corn meal (800 g) (twice a day)

The diets were supplied individually, immediately after milking (morning and late afternoon) and were laid over the corn silage, along the feeding trough, starting 10 days before the expected day of calving and extending up to 39 days postpartum. The same crude protein content was maintained in all of the partial substitution diets according to nutritional recommendations of NCR 2001 (*Nutrient Requirements of Dairy Cattle, National Academy Press*) (NCR, 2001).

Observations of follicular dynamics during puerperium

An ultrasound device (Sonoscape A6, linear transducer 5 to 11 MHz, China) was used to monitor ovarian activity during puerperium, focusing on mean follicle diameter (largest diameter + smallest diameter/2) (Gastal *et al*. (2008), between 6 and 39 days postpartum, with an interval of 2 to 3 days between examinations.

Statistical Analysis

The data were analyzed using Statistical Analysis System (SAS, version 9.1 for Windows, SAS Inst, Cary, NC, USA, 2014). The effects of different diets (SG, CUG, or SUG) on mean follicle diameter (mm) on each day of puerperium (d6 to d39) were evaluated using analysis of variance (ANOVA). Tukey’s test was used for multiple comparisons of means, at the 5% significance level, to evaluate the statistical differences between groups. In addition, the interaction between Groups × Days was evaluated; in the absence of interactions between factors, a day effect test was used to evaluate differences in the mean follicle diameter (mm) over time.

RESULTS AND DISCUSSION

Table 1 shows the mean diameter of the largest follicle in the SG, CUG, and SUG during puerperium (d6 to d39). Additional reproductive findings during the study were: four SG cows ovulated until the end of the period (on d39) and two had follicular cysts on d14, which regressed spontaneously in the following week.

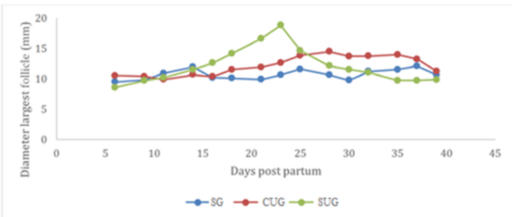
Table 1 - Mean of the diameter of the largest ovarian follicle (mm) during the puerperium of dairy cows submitted to daily diets with 900 g of soybean meal (SG); 130 g of conventional livestock urea (CUG) and 147 g of slow-release urea – SION (GUS) accompanied by ultrasound examinations at intervals of 2/3 days.

Days PP	SG (n=12)	CUG (n=16)	SUG (n=16)	*P* value
06	9,5 ± 2,22	10,5 ± 3,84	8,6 ± 3,18	0,30
09	9,8 ± 3,98	10,4 ± 3,80	9,7 ± 2,38	0,25
11	10,9 ± 3,02	9,9 ± 3,72	10,2 ± 2,98	0,42
14	11,95 ± 4,22	10,68 ± 6,90	11,51 ± 3,88	0,92
16	10,17 ± 2,91	10,36 ± 5,25	12,61 ± 3,38	0,79
18	10,08 ± 2,88	11,53 ± 4,00	14,20 ± 2,28	0,33
21	9,90 ± 3,63	11,94 ± 3,37	16,65 ± 3,08	0,08
23	$10{,}67 \pm 2{,}33^{b}$	$12{,}65 \pm 3{,}7^{b}$	$18{,}86 \pm 1{,}62^{a}$	<0,05
25	11,58 ± 1,62	13,86 ± 3,05	14,66 ± 3,95	0,44
28	10,65 ± 2,22	14,49 ± 3,90	12,16 ± 6,83	0,45
30	9,75 ± 3,67	13,72 ± 4,94	11,51 ± 1,09	0,38
32	11,21 ± 4,04	13,74 ± 3,04	11,06 ± 1,09	0,23
35	11,53 ± 4,02	13,99 ± 3,93	9,71 ± 3,48	0,28
27	12,11 ± 3,92	13,26 ± 3,60	9,71 ± 2,94	0,50
39	10,67 ± 1,75	11,25 ± 3,40	9,85 ± 2,40	0,38

Means with different letters in the same line present statistical difference by the Tukey test (5%). Days PP = days Postpartum; SG = control group (did not receive urea) (n = 12); CUG = conventional livestock urea group (n = 16); SUG = Sion urea group (n = 16).

This study partially substituted soybean meal with conventional or slow-release urea to provide an increased NPN source to cows during puerperium without damaging ovarian activity. No differences ($P > 0.05$) were observed among the diet groups in the mean diameter of the largest follicle between d6 and d21. However, there was a significant difference ($P < 0.05$) between diet groups on d23 postpartum. The SUG diet produced a larger mean follicle diameter than the SG or CUG groups. Between d16 and d25 postpartum can be observed that in the SUG group a daily growth rate of the largest follicle occurred, higher than the rate of other groups (range 0.09-0.91 mm in SG; 0.41-1.21 in CUG, and 1.59 -2.45 mm in the SUG). After d25, there was a decrease in mean diameter in all diet groups, except in CUG, possibly due to a growing DF (Table 1). Our hypothesis was that the slow-release urea had no negative effects (due the slow release and slow absorption) and could even optimize normal ovarian activity during the postpartum period.

From Graphic 1, it is evident that the SUG diet produced a daily growth rate of the largest follicle that was greater than that by other diet groups, showing a mean growth of 0.60 mm per day ($P < 0.05$) between d6 and d21, whereas the CUG presented a growth of 0.13 mm/day and the SG a growth rate of 0.07 mm/day.



Graphic 1 - Mean diameter (mm) of major ovarian follicle during the puerperium of dairy cows submitted to daily diets with 900 g of soybean meal (SG); 130 g of conventional livestock urea (CUG) and 147 g of slow release urea (SUG monitored by ultrasound examinations at intervals of 2/3 days.

A more detailed analysis of the follicular growth curves (Graphic 1) shows that SUG was the only group with positive mean daily growth rates until d23. An expressive follicular growth rate during puerperium precedes the return of ovarian cyclic activity. The pattern of follicle-stimulating hormone released during early puerperium determines the emergence of follicular waves; however, ovulation occurs when DFs reach largest diameters (Oliveira *et al*., 2010). The CUG produced negative mean daily growth rates on days 9, 11, and 16, and showed the largest variance in mean DF growth rate. The SG presented negative mean daily growth rates between d16 and d21.

The increase in follicular diameter occurs simultaneously with the growth of granulosa cells, while during follicular atresia, apoptosis occurs and a reduction in follicular diameter is observed (Rodgers and Irving-Rodgers, 2010). Costa *et al*. (2008) demonstrated a high correlation (r = 0.99) between the number of apoptotic cells and the plasma urea nitrogen concentration in dairy cows. It is possible that CUG presented lower rates of follicular growth due to the urea nitrogen in the blood plasma. High urea nitrogen content in the blood plasma affects the viability of bovine embryos younger than 7 days (Rhoads *et al.*, 2006). Therefore, it is possible that the increase in urea nitrogen level in the blood plasma is correlated with reduced fertility of dairy cows, affecting the quality of the oocyte, although the timing and physiological mechanisms are unknown.

There are deleterious effects on follicle and oocyte quality in dairy cows when they are fed diets supplemented with urea. However, it is not known exactly how much these changes influence follicular growth. It is likely that these changes occur only when there is an imbalance between the available urea and the protein synthesis capacity of the ruminal microbiota (Pinos-Rodrigues *et al.*, 2010).

Alternative urea-based commercial feed products (e.g., slow-release protected urea) are available. On the assumption that the slow-release urea administered in the feed did not exceed the protein metabolism capacity of the ruminal microbiota (resulting in a lower N-NH3 uptake via the rumen), no deleterious effects of elevated serum urea nitrogen levels were observed. In this study, the quality of the follicle or oocyte itself was not evaluated, only follicular growth in cows fed urea. Cherdthong and Wanapat (2010) reported that protected urea added to cow diets not only promoted a reduction in ruminal N-NH3, but also increased the microbial population compared to that in cows fed conventional livestock urea. Maggioni *et al*. (2008) point out a deleterious effect on the diameter of DF from diets that increased serum N-NH3. We believe that in this experiment the nutritional contribution provided by a urea-based diet (effectively multiplying the ruminal microbiota), may have influenced the growth rate of the largest follicle up to d25 postpartum.

Several variables affect follicular development; however, the influence of nutritional intake, BCS, and negative energy balance (NEB) during puerperium are highlighted (Oliveira *et al*., 2010). The use of food management strategies that reduce the effects of NEB can positively affect factors influencing fertility, including follicular development (Buttler and Smith, 1989; Lopez *et al.* 2004). Thus, the hypothesis of this study was confirmed, since substituting the soybean diet with urea (either slow-release or conventional) in the proportions given did not reduce ovarian activity in cows. A higher follicular growth rate was observed in cows fed with slow-release urea, which paves the way for further research into the physiological mechanisms behind resumed ovarian activity, and reduction of postpartum anestrous.

CONCLUSION

Partial substitution of soybean meal with conventional or slow-release urea can be performed without damage to ovarian follicular activity in dairy cows during puerperium; slow-release urea showed greater effects on ovarian activity than the other diets.

REFERENCES

AGUIAR, A.C.R.; OLIVEIRA, C.R.; CALDEIRA, L.A. et al. Consumo, produção e composição do leite e do queijo de vacas alimentadas com níveis crescentes de ureia. **Revista Brasileira de Ciência Veterinária**, v. 20, n.1, p. 37 – 42, 2013.

BUTLER, W.R.; SMITH, R.D. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. **Journal of Dairy Science**, v. 72, n. 3, p. 767 – 783, 1989.

CHERDTHONG, A.; WANAPAT, M. Development of urea products as rumen slow-release feed for ruminant production: A review. **Australian Journal ou Basc and Applied Sciences**, v. 4, n. 8, p. 2232 – 2241, 2010.

COSTA, S.M.; CHAVEIRO, A.; ANDRADE, M. Efeito da ureia na ativação da apoptose em células da granulosa de bovinos avaliada por citometria de fluxo. **Revista Portuguesa de Ciências Veterinárias**, v. 103, p. 41 – 45, 2008.

DAWUDA, P.M.; SCARAMUZZI, R.J.; LEESE, H.J. et al. Effect of timing of urea feeding on the yield and quality of embryos in lactating dairy cows. **Theriogenology**, v. 58, n. 8, p. 1443 – 1455, 2002.

FERGUSON, J.D.; BYERS, D.; FERRY, J. et al. Round table discussion: body condition of lactating cows. **Agri-Practice**, v. 15, n. 4, p. 17 – 21, 1994.

GASTAL, E.L.; NEVES, A.P.; MATTOS, R.C. et al. Miniature ponies: 1. Follicular, luteal and endometrial dynamics during the oestrous cycle. **Reproduction, Fertility and Development**, v. 20, p. 376 – 385, 2008.

GRANT, J.K.; STEICHEN, P.L.; WRIGHT, C.L. et al. Influence of nitrogen and sulfur intake on bovine uterine pH throughout the luteal phase. **Journal of Animal Science**, v. 91, n. 3, p. 1186 – 1192, 2013.

HAMMON, D.S.; HOLYOAK, G.R.; DHIMAN, T.R. Association between blood plasma urea nitrogen levels and reproductive fluid urea nitrogen and ammonia concentrations in early lactation dairy cows. **Animal Reproductive Science**, v. 86, p. 95 – 204, 2005.

IMAIZUMI, H.; SANTOS, F.A.P.; BITTAR, C.M.M. et al. Diet crude protein content and sources for lactating dairy cattle. **Scientia Agricola**, v. 67, p. 16 – 22, 2010.

LAPIERRE, H.; LOBLEY, G.E. Nitrogen recycling in the ruminant: a review. **Journal of Dairy Science**, v. 84, n.1, p. 223 – 236, 2001.

LAVEN, R.A.; DAWUDA, P.M.; SCARAMUZZI, R.J. et al. The effect of feeding diets high in quickly degradable nitrogen on follicular development and embryo growth in lactating Holstein dairy cows. **Animal Reproduction** Science, v. 84, n. 1 – 2, p. 41 – 52, 2004.

LEROY, J.L.M.R.; VANHOLDER, T.; DELANGHE, J.R. et al. Metabolic changes in follicular fluid of the dominant follicle in high-yealding dairy cows early postpartum. **Theriogenology**, v. 62, p. 1131 – 1143, 2004.

LOPEZ, H.; SATTER, L.D.; WILTBANK, M.C. Relationship between level of milk production and estrous behavior of lactating dairy cows. **Animal Reproduction Science**, v. 81, n. 3-4, p. 209 – 223, 2004.

MAGGIONI, D.; ROTTA, P.P.; MARQUES, J.A. et al. Influência Da Proteína Sobre a Reprodução Animal: Uma Revisão. **Campo Digital**, v. 1, n. 2, p. 105 – 110, 2008.

NATIONAL RESEARCH COUNCIL (NRC). **Nutrient requirements of dairy cattle**. 7.ed. Washington: National Academy Press; 2001.

OLIVEIRA, J.F.C. DE; GONÇALVES, P.B.D.; FERREIRA, R. et al. Controle sobre GnRH durante o anestro pós-parto em bovinos. **Ciência Rural**, v. 40, p. 2623 – 2631, 2010.

PINOS-RODRIGUEZ, J.M.; PEÑA, L.Y.; GONZALEZ-MUÑOZ, S.S. et al. Effects of a slow-release coated urea product on growth performance and ruminal fermentation in beef steers. **Italian Jounal of Animal Science**, v. 9, n. 1, p. 16 – 19, 2010.

RHOADS, M.L.; RHOADS, R.P.; GILBERT, R.O. et al. Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. **Animal Reproduction Science**, v. 91, n. 1-2, p. 1 – 10, 2006.

RODGERS, R.J.; IRVING-RODGERS, H.F. Morphological classification of bovine ovarian follicles. **Reproduction**, v. 139, n. 2, p. 309 – 318, 2010.

SOUZA, V.L.; ALMEIDA, R.; SILVA, D.F.F. et al. Substituição parcial de farelo de soja por ureia protegida na produção e composição do leite. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 62, n. 6, p. 1415 – 1422, 2010.

SOUZA, V.M.; CALDEIRA, L.A.; RIBEIRO, V. et al. Efeito de níveis crescentes de ureia na alimentação de vacas sobre o rendimento, composição, perfil de ácidos graxos e sensorial do queijo minas frescal. **Revista Brasileira de Ciência Veterinária**, v. 22, n. 2, p. 107 – 113, 2015.