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The effect of diets with different levels of degradable and undegradable protein in rumen on apoptosis in oocyte and in cumulus-oophurus cells of Girolando cows

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The ammonia produced during rumen protein degradation and not used in microbial protein synthesis, is absorbed by the rumen wall and converted to urea in the liver. High serum concentrations of ammonia and urea cause increase of both in tissue and reproductive fluids, interfering negatively in the fertility and in vitro embryos production. This study evaluated the effect of diets with different levels of degradable protein (RDP) and undegradable protein in rumen (RUP) on apoptosis of oocytes and cumulus-oophorus cells in crossbred Girolando cows. 22 Girolando cows were evaluated (n=10 3/4 HG; n=12 7/8 HG) with average weight 475.8 \pm 7.75 kg, BCS 3.22 \pm 0.03 and 105.33 \pm 23.15 days of postpartum. The animals were distributed in four experimental groups, fed twice daily for 68 days with a total diet based on corn silage and concentrate. The diet of each group varied in the relation between RDP:RUP, with maintenance of metabolizable protein (1888g/day) and reduction of crude protein (CP) (RDP:RUP1.68= 15.4% CP, 62.7% RDP, 37.3% RUP; RDP:RUP 1.31= 13.6% CP, 56.7% RDP, 43.3% RUP; RDP:RUP 1.08= 13% CP, 52% RDP, 48% RUP; RDP:RUP0.83= 12.4% CP, 45.4% RDP, 54.6% RUP). OPU's were done on days 33 and 63 after initiation of treatment. The follicular waves were synchronized at 72 hours before by puncture of all follicles present in the ovaries. The cumulus-oocyte complex (COC) were recovered in PBS medium with 20 UI/mL of heparin, classified as viable (grades 1, 2 and 3) and non-viable. The COC viable were fixed in formalin 10%, alcohol PVA, grouped by treatment, collection day and stained with DAPI and TUNEL (Promega, Wisconsin, USA). The structures were photographed in fluorescence confocal microscope Leica TCS SP5II (Leica Microsystems®, Wetzlar, Germany) at 40x magnification. Images obtained at each 16µm were evaluated in Leica LAS AF Lite software. Getting the number and rates of the cumulus apoptotic cells and the percentage of apoptotic oocytes. The variables were submitted to analysis of variance, using a generalized linear model (PROC GLM) and means were compared using the Student t test (P<0.05). Were recovered 78 COC (RDP:RUP1.68= 3; RDP:RUP1.31= 19; RDP:RUP1.08= 21; RDP:RUP0.83= 35). No significant effect was found in the number of apoptotic cumulus cells (RDP:RUP1,68= 9.33±15.3; RDP:RUP1.31= 40.94±9876; RDP:RUP1.08= 11.12 ±20.0; RDP:RUP0.83= 4.35 ±10.11) and rates cumulus cell apoptotic (RDP:RUP1.68= 0.027±0.02; RDP:RUP1.31= 0.1±0.16; RDP:RUP1.08= 0.12±0.14; RDP:RUP0.83= 0.03±0.1), but there were effect on the percentage of apoptotic oocytes (RDP:RUP1.68= 0%ab; RDP:RUP1.31= 26.32%a; RDP:RUP1.08= 4.76%ab; RDP:RUP0.83= 0%b). The variation in relation between RDP:RUP in diet interfered in apoptosis of oocytes, however, the highest ratio RDP:RUP not different from the two smaller relations.

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