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PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA CONVENCIONAL E INTEGRATIVA
Rodovia Ulysses Gaboardi, km3 - CEP: 89.520-000 – Cx. Postal 101
Curitibanos – Santa Catarina – Brasil – CEP 89520-000
https://ppgmvci.ufsc.br/ Tel.: (48) 3721-6273 E-mail: ppgmvci@contato.ufsc.br

Avaliação Escrita

EDITAL N° 03/2020/PPGMVCI

Cada candidato deverá responder, obrigatoriamente, um total de **QUATRO QUESTÕES**, de livre escolha dentre as 6 questões da prova. Caso sejam respondidas mais que quatro questões, serão corrigidas apenas as 4 primeiras respostas na ordem que aparecerem no arquivo de resposta.

As respostas, devem ser redigidas em Língua Portuguesa, deverão ser devolvidas **até às 14:00** horas do mesmo dia (22/02/2021 - horário de Brasília) por e-mail (sipg.ppgmvci@contato.ufsc.br) informando no assunto "Seleção Mestrado - nome do candidato".

As respostas deverão seguir as normas abaixo:

• A avaliação deverá conter um cabeçalho apresentando unicamente o número do edital e o número de inscrição do candidato. **As avaliações NÃO deverão ser nominadas**. Avaliações nominadas levarão à desclassificação do candidato.

• As respostas deverão ser encaminhadas em **um arquivo único, identificado com o número da inscrição do candidato**. O conjunto das respostas deverá estar contido em no **MÁXIMO 6 PÁGINAS**, tamanho A4, com margens de 2 cm, letra tipo *Times New Roman* tamanho 12 e espaçamento 1,5 cm; gravado em formato de extensão ".pdf". Avaliações que não estiverem em formato PDF levarão à desclassificação do candidato.

• **As provas deverão conter ao final a seguinte declaração de inexistência de plágio**: "Declaro que o texto acima é original, de minha autoria, não contendo material copiado no todo ou em parte de quaisquer outras fontes, sem a devida referência". As avaliações serão verificadas utilizando-se um *software* anti-plágio, o qual se constatado levará à desclassificação do candidato.

QUESTÃO 1:

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—Original Article—

L-carnitine prevents bovine oocyte aging and promotes subsequent embryonic development

Wen-Jie JIANG[1, 2], Xue-Rui YAO[1, 2], Yu-Han ZHAO[1, 2], Qing-Shan GAO[1, 2], Qing-Guo JIN[1, 2], Ying-Hua LI[1, 2], Chang-Guo YAN[1, 2] and Yong-Nan XU[1, 2]

[1)] *College of Agriculture, Yanbian University, Yanji 133000, China*
[2)] *Engineering Research Center of Yanbian Yellow Cattle Resources Reservation, Yanji 133000, China*

Introduction

Mammalian oocytes are arrested in the metaphase of the second meiosis (MII) phase, where they await fertilization. If no fertilization occurs within an appropriate time, the quality of oocytes gradually deteriorates, a process termed as “postovulatory aging” [1]. In humans and livestock, it is well known that postovulatory aging of oocytes may affect the results of assisted reproductive technologies (ARTs), such as artificial insemination [2], *in vitro* fertilization (IVF) [3, 4], and intracytoplasmic sperm injection [5, 6]. In bovine, both *in vivo* and *in vitro* aging of oocytes can result in reduced fertilization and embryonic development [2, 3, 7–10]. Extensive research on aged bovine oocytes may help in the development of a method to prevent aging in matured bovine oocytes, resulting in improved efficacy of ARTs.

It has been demonstrated that, following ovulation, intracellular reactive oxygen species (ROS) accumulation increases in oocytes with time [11, 12]. Oocytes exhibit an intracellular defense [(via the antioxidant glutathione (GSH)] mechanism against an oxidative attack. However, this defense response decreases with aging after ovulation [13]. Thus, aging oocytes after ovulation undergo oxidative stress due to an increase in ROS level, and a decrease in antioxidant defenses, causing multiple oxidative damages in cell structures, including lipid peroxidation of membranes, enzyme inactivation, protein oxidation, and DNA damage [14, 15]. The imbalance between ROS and their normal scavenger antioxidants leads to oxidative stress, which adversely affects embryonic development through structural and functional alterations. Increased production of ROS in aging oocytes reduces intracellular ATP concentration [16] and glutathione disulfide ratio [17–19]. This outcome adversely affects fertilization and subsequent embryonic development, thereby increasing the risk of an early miscarriage and abnormal development of offspring [20, 21].

L-carnitine (LC), the biologically active form of carnitine (3-hydroxy-4-N-trimethyl amino butyrate, $C_7H_{15}NO_3$), is a naturally occurring, vitamin-like water-soluble quaternary ammonium compound. It is mainly synthesized from the amino acids lysine and methionine, in the liver. LC is required to transport fatty acids from the cytosol to the mitochondria during the breakdown of lipids (fats), to generate metabolic energy. As an antioxidant, LC neutralizes free radicals, especially superoxide anions, and protects cells from oxidative damage-induced apoptosis [22]. Although the effects of LC on the *in vitro* development of bovine embryos [23], pig embryos [24], and mouse embryos [25] have been previously reported, there are no reports regarding the effects of LC on aging bovine oocytes.

The best mature culture period for bovine embryo production is 20–22 h. Upon extension of this period, the blastocyst formation rate relatively decreases [3]. Previous studies have considered bovine oocytes at about 30 h after *in-vitro* maturation (IVM), as aged or slightly aged and used them to investigate age-related changes [8, 26, 27]. Oocytes after 30 h of IVM showed a low blastocyst development rate [8].

In the present study, aging bovine oocytes treated with LC were evaluated for ROS and GSH levels, mitochondrial membrane potential ($\Delta\Psi$m), early apoptosis levels, and caspase-3 activity indicators in order to identify whether LC treatment improved the performance of oocytes. The aim of this study was to investigate the potential of LC in delaying aging via reducing oxidative stress in bovine oocytes.

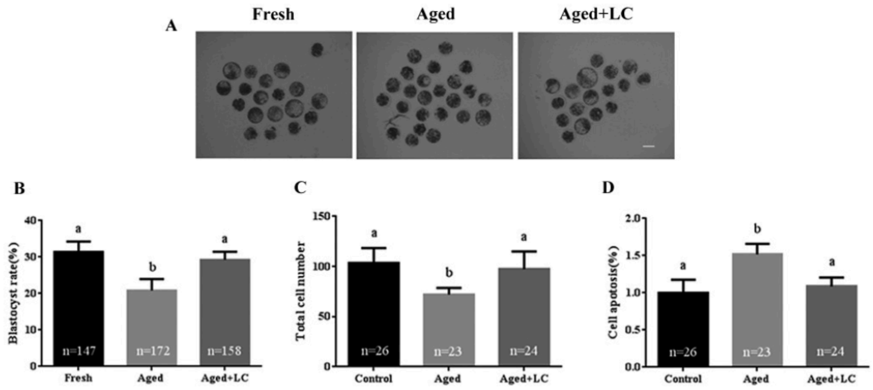

Fig. 1. Effect of L-carnitine (LC) on the development and quality of aged bovine oocytes *in vitro*. (A) Blastocyst formation on day 7. Scale bar: 100 µm. (B) Blastocyst rate. R = 5. (C) Total cell number in each day-7 blastocyst. R = 3. (D) The rate of cell apoptosis in the day-7 blastocysts, R = 3. Statistically significant differences are represented with different letters (P < 0.05).

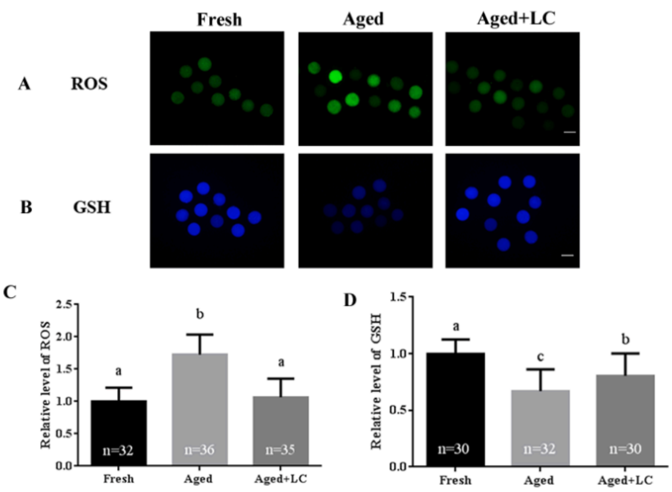

Fig. 2. Effect of L-carnitine (LC) on ROS and GSH levels in aged bovine oocytes *in vitro*. (A) Oocytes were stained with H2DCFDA to detect the intracellular levels of ROS. Scale bar: 100 µm, R = 3. (B) Oocytes were stained with Tracker Blue CMF2HC dye to detect the intracellular levels of GSH. Scale bar: 100 µm, R = 3. (C) and (D) The relative intracellular levels of ROS and GSH in bovine oocytes from the three groups (fresh, aged, and aged + LC). Statistically significant differences are represented with different letters (P < 0.05).

A figura 1 demonstra a taxa de desenvolvimento embrionário (B), número total de células (C), e apoptose nos embriões dos diferentes grupos experimentais. (*Fresh=Grupo controle, Aged= oócitos envelhecidos com maior tempo de maturação, Aged+LC= oócitos envelhecidos tratados com L-carnitine*)

a) **Descreva os resultados demonstrados nas figuras 1 e 2: (1,5 pontos)**

b) **Você concorda com a premissa de que o tratamento com *L-carnitite* tem efeito positivo sobre o desenvolvimento embrionário e níveis de ROS e GSH, justifique sua resposta baseado na análise dos resultados. (1,0 ponto)**

QUESTÃO 2:

Effects on cattle of transportation by road for up to 31 hours

T. G. KNOWLES, P. D. WARRISS, S. N. BROWN, J. E. EDWARDS

The physiological and behavioural effects on cattle of transporting them for periods of 14, 21, 26 and 31 hours, including a stop for a rest and drink on the lorry after 14 hours, were studied in 120 transported animals and 48 control animals. The physiological measurements indicated that a journey lasting 31 hours was not excessively physically demanding, but many of the animals chose to lie down after approximately 24 hours. The animals that lay down had higher plasma cortisol levels than those that remained standing. Many animals chose not to drink during the rest stop. Physiological measurements made after the journeys indicated that 24 hours in lairage, with hay and water freely available, allowed the animals to recover substantially, although not completely, irrespective of the journey time.

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145, 575-582

T. G. Knowles, BSc, MSc, PhD,
P. D. Warriss, BSc, PhD, FIBiol, FIFST,
S. N. Brown, MIBiol,
J. E. Edwards, MRIPHH,
School of Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU

THE latest European Union rules (Anon 1991), implemented in the UK as the Welfare of Animals (Transport) Order 1997, allow mature cattle to be transported by road for up to 31 hours, provided that they are given a one-hour rest with water and, if necessary, feed, after the first 14 hours. However, work by Warriss and others (1995) has indicated that journeys of this length could compromise the welfare of the animals. It provided evidence that although 15 hours of road transport was acceptable in terms of animals' welfare, the cattle were becoming physically fatigued by the end of the journey, probably because they preferred to remain standing and did not lie down and rest.

The purpose of this study was to investigate the responses of mature cattle transported under the conditions and limitations of the newly introduced EU legislation and to evaluate the implications of the new rules in terms of the animals' welfare. The response of the animals during journeys of up to 31 hours and during the first 72 hours of recovery was studied by means of physiological measurements and behavioural observations.

MATERIALS AND METHODS

Three replicate transport experiments were carried out, starting on April 28, June 30 and July 28, 1997, in each of which, 42 mature beef cross steers and heifers of mixed breeds and of mean weight 571·5 kg, purchased from local auction markets, were used. A control study was also carried out, starting on May 26, 1997, which was identical in sampling structure and design, but in which the cattle remained in their home pens with access to food and water; its purpose was to provide information on the effects of blood sampling the animals, and the associated handling and disruption to their normal patterns of behaviour.

For each experiment, the animals were purchased between 14 and eight days before the journeys and kept at the University of Bristol farm to allow them time to rest and recover. Seven days before the journeys, the animals were weighed and blood samples were taken to provide baseline values. They were then randomly allocated into five covered, strawed pens in four groups of 10 and one group of two; the allocations were stratified by weight. The cattle had constant access to water and hay (87 per cent dry matter, 12·5 per cent total crude protein, 36·3 per cent modified acid digestible fibre) and received 1 kg per animal per day of concentrate (Growergrain nut 16 per cent protein; BOCM Pauls).

On the day of the journeys, the four groups of 10 animals were randomly allocated to be transported for 14, 21, 26 or 31 hours and the 10 animals in each group were randomly allocated, stratified by weight, to one of two types of lorry, a single-deck articulated cattle lorry with leaf spring suspension, or a double-deck articulated cattle lorry with air suspension. Thus, each lorry contained four pens of five animals which were to be transported for 14, 21, 26 or 31 hours. The pens were well bedded with wood shavings. At 08.00 on the day of the journey the 40 cattle were loaded on to the lorries at an approximate space allowance of 1·55 m² per animal and the journeys began. The two remaining animals were loaded into a small farm cattle trailer and driven half a mile to the University of Bristol's slaughterhouse for immediate slaughter. At 14, 21, 26, and 31 hours after the journey began, both lorries returned to the university farm, having first unloaded one animal, chosen at random from the appropriate pen, at the university slaughterhouse for immediate slaughter. At the farm, the remaining animals were unloaded and the lorries

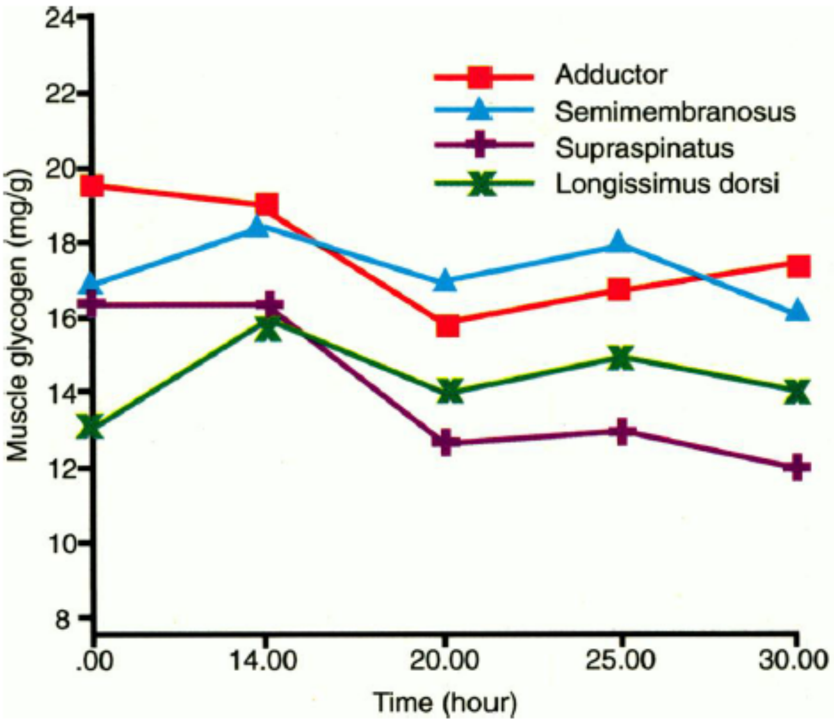


FIG 2: Changes in muscle glycogen with increasing journey time within each of the four muscles

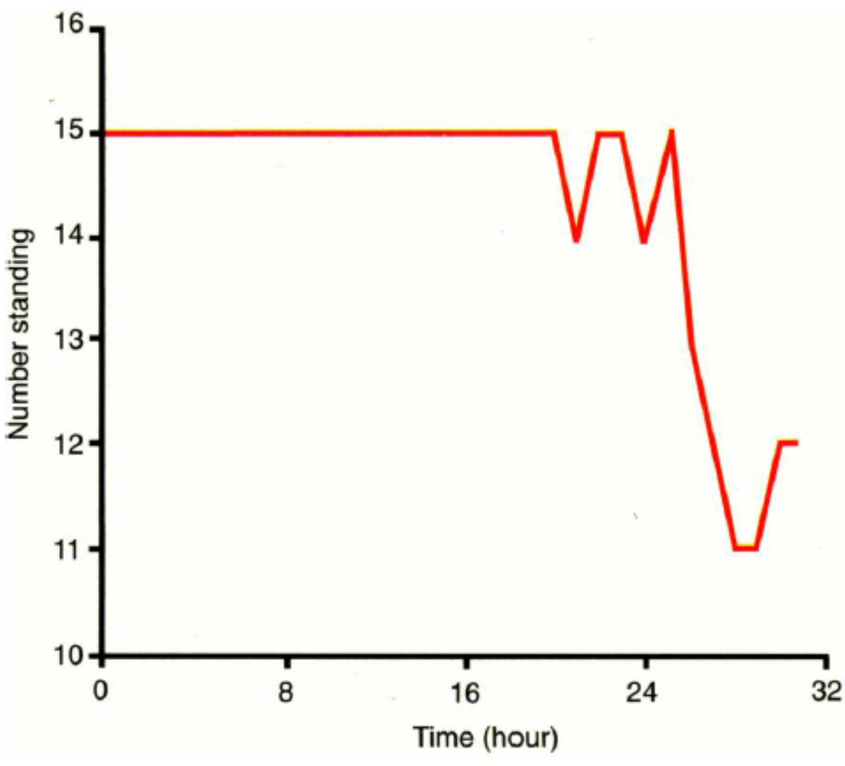
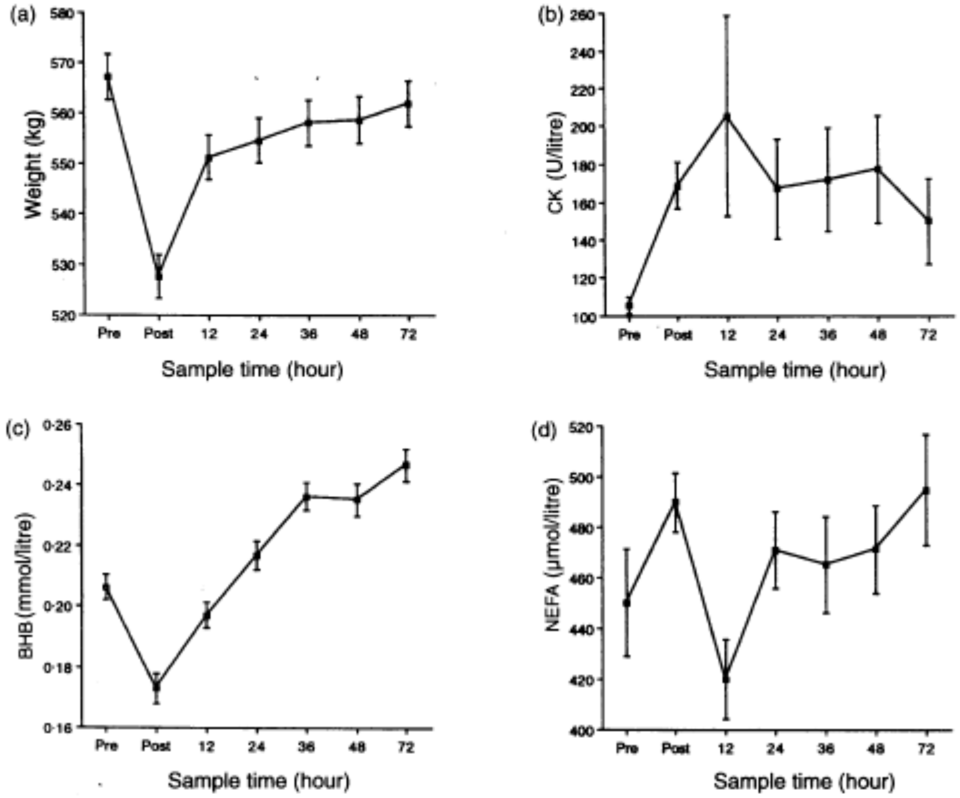


FIG 7: Number of animals transported for 31 hours on the single-deck lorry which were standing up during the journey

a) **Com base nos gráficos acima, determine qual o melhor tempo médio de viagem para os animais, justifique a sua resposta. (1,0 ponto)**



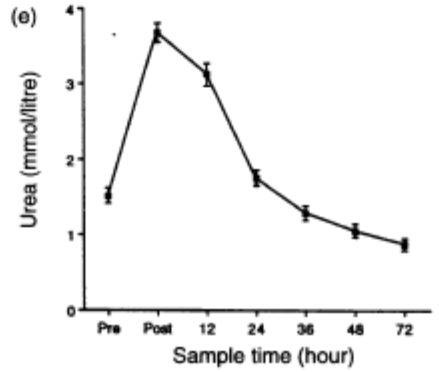


FIG 5: Covariate-adjusted mean (se) (a) liveweight, (b) plasma creatine kinase (CK), (c) beta-hydroxbutyrate (BHB), (d) non-esterified fatty acids (NEFA) and (e) urea for all the groups during the recovery period. Before the journeys n=120 and after the journeys n=72. The horizontal axis is not to scale

b) No mesmo estudo algumas covariáveis foram observadas de acordo com os gráficos acima. Do ponto de vista homeostático, explique o que aconteceu com esses animais durante a viagem e após a mesma (1,5pontos).

QUESTÃO 3:

As tabelas abaixo apresentam os resultados publicados de um ensaio que avaliou o efeito de diferentes níveis de fitases e multicarboidrases na dieta de frangos de corte, formulada com redução dos níveis de cálcio, fósforo, proteína bruta e energia metabolizável (controle negativo), em comparação com dieta formulada para atender as exigências nutricionais e energéticas, sem a inclusão dos aditivos (controle positivo).

a) Com bases nos dados apresentados nas tabelas abaixo é correto afirmar que aves alimentadas com dieta com inclusão de fitase e multicarboidrases apresentam maior ganho de peso, maior consumo de ração e melhor conversão alimentar? Justifique sua resposta. (0,9 pontos)

b) Com bases nos dados apresentados nas tabelas abaixo é possível afirmar que o aumento dos níveis de inclusão de fitases e multicarboidrases associadas na dieta das aves, melhora a mineralização óssea? Avalie o comportamento das variáveis e justifique sua resposta. (0,8 pontos)

c) Quais conclusões que podem ser extraídas da Tabela 4, em relação ao uso isolado de fitase sobre a retenção de nitrogênio e digestibilidade ileal do fósforo? Justifique sua resposta. (0,8 pontos)

Table 2. Effects of phytase and multicarbohydrase on the growth performance of broilers fed a nutritionally reduced diet.

Items	Phytase[1] (FTU/kg)	MC[1,2] (mg/kg)	0 to 7 d BWG (g)	0 to 7 d FI (g)	0 to 7 d FCR	0 to 14 d BWG (g)	0 to 14 d FI (g)	0 to 14 d FCR	0 to 18 d BWG (g)	0 to 18 d FI (g)	0 to 18 d FCR
Treatments											
PC			105	117	1.122	357	488	1.365	$563^{a,b}$	799	1.419^{b}
NC[2]			100	116	1.161	338	478	1.427	516^{b}	779	1.514^{a}
3	500	0	106	122	1.159	358	504	1.410	$549^{a,b}$	812	$1.483^{a,b}$
4	1,000	0	114	125	1.099	365	502	1.384	$560^{a,b}$	805	$1.438^{a,b}$
5	1,500	0	105	115	1.085	390	501	1.298	$564^{a,b}$	790	1.406^{b}
6	500	500	107	119	1.121	378	498	1.320	577^{a}	805	1.395^{b}
7	1,000	500	118	135	1.144	393	534	1.359	596^{a}	852	$1.430^{a,b}$
8	1,500	500	106	117	1.105	364	498	1.371	579^{a}	817	1.414^{b}
SEM[3]			5.05	5.41	0.019	12.26	15.86	0.033	14.58	20.04	0.029
P-value			0.370	0.276	0.076	0.300	0.499	0.218	0.032	0.412	0.024
Linear phytase			0.312	0.966	0.002	0.093	0.377	0.013	0.026	0.788	0.006
Quadratic phytase			0.200	0.151	0.768	0.665	0.432	0.323	0.332	0.256	0.987
Linear phytase + MC[1,2]			0.222	0.426	0.111	0.159	0.210	0.400	0.005	0.112	0.040
Quadratic phytase + MC[1,2]			0.101	0.085	0.959	0.023	0.111	0.096	0.016	0.174	0.080

[a,b]Means with different superscripts within each column are significantly different ($P < 0.05$).

Abbreviations: FTU, phytase unit; MC, multicarbohydrase; NC, negative control; PC, positive control.

[1]Bio-phytase (5,000 FTU/kg) and Superzyme-CS (Xylanase 1,300 u/g; beta-Glucanase 150 u/g; cellulase 800 u/g; alpha-Amylase 12,000 u/g; protease 6,000 u/g; mannanase 5 u/g; invertase 700 u/g.) from Canadian Bio-System Inc.

[2]A reduction of Ca, nPP, CP, and ME by 0.15%, 0.15%, 1.5%, and 75 kcal/kg, respectively, compared with the PC diet.

[3]Means are from 6 replicates of 10 birds each at the beginning of study.

Table 3. Effects of phytase and multicarbohydrase on the bone mineralization of broilers fed a nutritionally reduced diet.

Items	Phytase[1] (FTU/kg)	MC[1] (mg/kg)	Tibia ash: Ash weight (mg)	Tibia ash: Ash percentage (%)	Tibia ash: Concentration[2] (mg/cubic cm)
Treatments					
PC			0.627[a]	44.67[a]	0.210[a]
NC[3]			0.408[c]	40.30[b]	0.144[c]
3	500	0	0.503[b,c]	43.62[a]	0.170[b]
4	1,000	0	0.528[a,b,c]	44.44[a]	0.178[b]
5	1,500	0	0.519[a,b,c]	44.39[a]	0.183[b]
6	500	500	0.523[a,b,c]	43.83[a]	0.174[b]
7	1,000	500	0.603[a,b]	46.01[a]	0.194[a,b]
8	1,500	500	0.555[a,b]	45.69[a]	0.184[a,b]
SEM[4]			0.035	0.720	0.008
P-value			0.010	0.004	0.001
Linear phytase			0.037	0.002	0.003
Quadratic phytase			0.167	0.069	0.223
Linear phytase + MC[1,2]			0.003	<0.001	<0.001
Quadratic phytase + MC[1,2]			0.042	0.049	0.036

[a–c]Means with different superscripts within each column are significantly different ($P < 0.05$).

Abbreviations: FTU, phytase unit; MC, multi-carbohydrase; NC, negative control; PC, positive control.

[1]Bio-phytase (5,000 FTU/kg) and Superzyme-CS (Xylanase 1,300 u/g; beta-Glucanase 150 u/g; cellulase 800 u/g; alpha-Amylase 12,000 u/g; protease 6,000 u/g; mannanase 5 u/g; invertase 700 u/g.) from Canadian Bio-System Inc.

[2]Ash concentration was calculated as ash weight/bone volume, and bone volume was calculated with the assumption that the specific gravity of water is 1 g/cubic cm.

[3]A reduction of Ca, nPP, CP, and ME by 0.15%, 0.15%, 1.5%, and 75 kcal/kg, respectively, compared with the PC diet.

[4]Means are from 6 replicates of 10 birds each at the beginning of study.

Table 4. Effects of phytase and multicarbohydrase on the nutrient digestibility of broilers fed a nutritionally reduced diet.

Items	Phytase[1] (FTU/kg)	MC[1] (mg/kg)	Nitrogen retention (%)	AMEn (kcal/kg)	Ileal P digestibility (%)
Treatments					
PC			67.2[a]	2,967[a]	49.46[b]
NC[2]			58.0[b]	2,759[b]	52.30[b]
3	500	0	67.8[a]	2,990[a]	63.64[a]
4	1,000	0	64.0[a]	2,954[a]	70.76[a]
5	1,500	0	66.0[a]	2,878[a]	69.76[a]
6	500	500	64.3[a]	2,929[a]	66.00[a]
7	1,000	500	66.8[a]	2,958[a]	66.58[a]
8	1,500	500	67.0[a]	2,972[a]	74.51[a]
SEM[3]			1.725	12.42	3.517
P-value			0.023	0.004	0.002
Linear phytase			0.021	0.079	<0.001
Quadratic phytase			0.059	0.054	0.084
Linear phytase + MC[1,2]			0.002	<0.001	<0.001
Quadratic phytase + MC[1,2]			0.122	0.066	0.479

[a,b]Means with different superscripts within each column are significantly different ($P < 0.05$).

Abbreviations: FTU, phytase unit; MC, multi-carbohydrase; NC, negative control; PC, positive control.

[1]Bio-phytase (5,000 FTU/kg) and Superzyme-CS (Xylanase 1,300 u/g; beta-Glucanase 150 u/g; cellulase 800 u/g; alpha-Amylase 12,000 u/g; protease 6,000 u/g; mannanase 5 u/g; invertase 700 u/g.) from Canadian Bio-System Inc.

[2]A reduction of Ca, nPP, CP, and ME by 0.15, 0.15, 1.5%, and 75 kcal/kg, respectively, compared with the PC diet.

[3]Means are from 6 replicates of 10 birds each at the beginning of study.

QUESTÃO 4:

As tabelas e gráficos abaixo são referentes aos resultados de uma pesquisa visando comparar a produtividade de rebanhos leiteiros que utilizavam pastejo rotacionado com o fornecimento constante de água nos piquetes e o fornecimento restrito de água em Santa Catarina (*Readily available water access is associated with greater milk production in grazing dairy herds*). Com base nessas informações e nas legendas das tabelas e gráficos, responda:

a) **Baseado com o descrito no texto abaixo, complementado pela tabela 2 (abaixo) e no gráfico da figura 1 (abaixo), os autores conseguiram comprovar a sua hipótese de que o fornecimento irrestrito de água proporcionaria um ambiente em que a produção diária de leite seria significativamente maior do que aquelas propriedades onde os animais não têm acesso a água durante o pastejo? Explique sua resposta. (0,25 a primeira parte da pergunta e 0,5 a explicação)**

3.2. Final Model

All variables used in the final model are presented in Table 2. Significant variables and their adjusted means based on the final model are presented in Figures 1 and 2. Provision of unrestricted access to water was associated with a higher average milk yield per cow/d (1.7 L; 95% CI = 3.3–0.2; $p = 0.03$; Figure 1A) compared to restricted water access. This represents a difference of 10% in milk

production between herds provided with unrestricted (adjusted mean: 18.1 L/cow/d) and restricted (adjusted mean: 16.4 L/cow/d) access to water. As expected there was an association between herd breed and milk production. Holstein herds produced on average 18% more milk per day per cow/d (2.8 L; 95% CI = 1.1–4.4; $p < 0.01$; Figure 1B) compared to non-Holstein herds (Holstein herds adjusted mean: 18.7 L/cow/d; non-Holstein herds adjusted mean: 15.9 L/cow/d). Amount of concentrate offered per cow/d was positively associated with average milk yield per cow/d (1.1 L increase in milk production/additional kg of concentrate offered/cow/d; CI = 0.6–1.6, $p < 0.01$; Figure 2). Area of silage/cow/yr was not significant (95% CI = −1.9–10.1, $p = 0.18$). The model explained 55% (adjusted R^2) of the variation in average milk yield per cow/d.

In conclusion, providing unrestricted access to water while grazing was associated with higher milk production, regardless of the breed of cows and the amount of concentrate and silage offered. Water availability should be considered when planning dairy management systems, as restricted water access may decrease milk production.

Table 2. Description of variables included in the final model [1].

Variable	Type	n^2	Mean	SD	Median	Min	Max
Herd average milk yield (kg/cow/d)	continuous	51	17.7	4.0	17.8	8.7	27.1
Silage area -hectare/cow/yr	continuous	51	0.27	0.13	0.24	0.10	0.77
Concentrate -kg/cow/d	continuous	50	5.2	1.6	5.0	1.5	9.0
Breed [3]	categorical	53					
Holstein		*26*					
non-Holstein		*27*					
Water provision [4]	categorical	52					
Restricted		*24*					
Unrestricted		*27*					

[1] In total five herds had incomplete data, thus only 48 herds were included in the model. [2] Deviations from 53 in the sum of observations per variable is due to missing values. [3] Holstein (herds >75% Holstein cows); non-Holstein (herds ≤ 75% Holsteins). [4] Restricted: paddocks without water trough and gate stayed closed. Unrestricted: herds that had one water through per grazing paddock and/or paddock gate stayed open. Italics and indentation reflect the levels of the categorical variable.

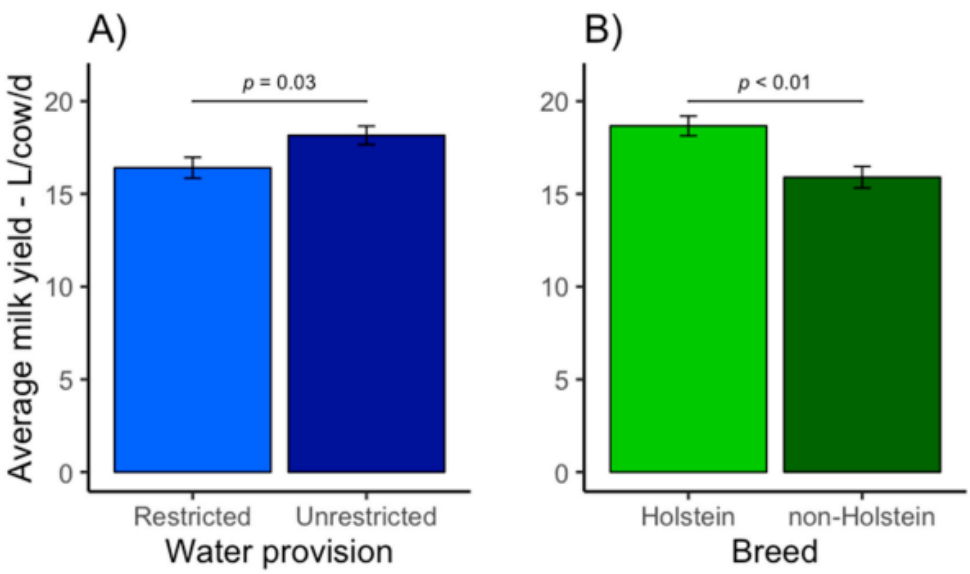


Figure 1. The effects of water provision (**A**) and breed (**B**) on daily milk production (L/cow/d) on grazing dairy herds (n = 48) in Santa Catarina State, Brazil. Means are presented as adjusted means and standard errors.

b) Baseado no gráfico da figura 1 (acima), a variação racial nesta pesquisa teve sua suposição inicial comprovada, onde esperava-se que vacas da raça holandesa teriam uma maior produção no sistema de pastejo rotacionado quando comparadas a outras raças? Explique a sua resposta e compare esta diferença com a diferença da forma de provisão de água referente a alternativa "1.1". (0, 25 primeira parte da pergunta e 0,25 a explicação)

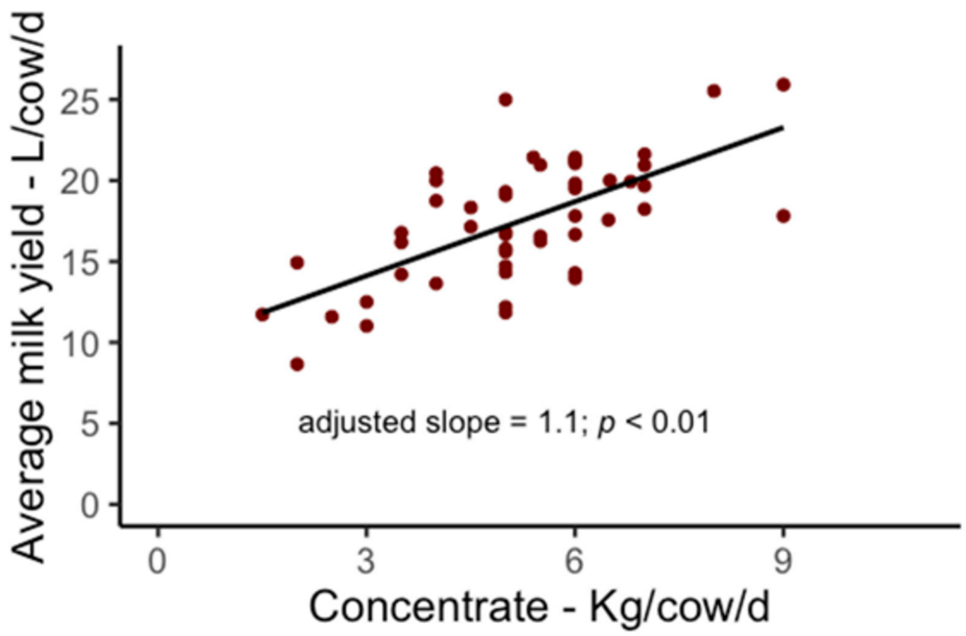


Figure 2. The effects of daily concentrate allocation per cow (kg/cow/d) on daily milk production (L/cow/d) on grazing dairy herds (n = 48) in Santa Catarina State, Brazil. Dots represent the raw values summarizing the average of each farm.

c) Baseado no gráfico da figura 2 (acima), explique a correlação entre os eixos. (0,5 pontos)

d) Sugira alguma alteração na metodologia deste trabalho para confirmar a hipótese deste estudo sobre o fornecimento de água irrestrito versus restrito na produção leiteira: (0,75 pontos)

QUESTÃO 5:

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Histochemistry of Equine Damaged Tendons, Ligaments and Articular Cartilage

Grasiela De Bastiani[1], Flávio Desessards De La Côrte[2], Karin Erica Brass[2], Camila Cantarelli[2], Stefano Dau[2], Glaucia Denise Kommers[3], Taiara Müller Da Silva[3] & Marcos Da Silva Azevedo[4]

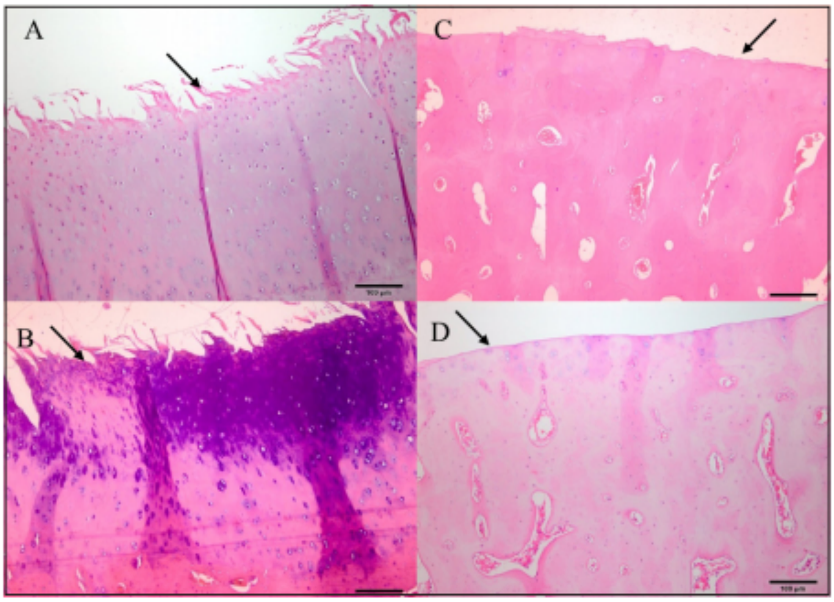


Figure 3. Articular Cartilage longitudinal section of the MCIII condyle. A- Fibrillation of the articular cartilage (arrow) [H&E, 100x]. B- Decrease of blue stained in fibrillation of articular cartilage (arrow) [Alcian blue stain, 100x]. C- Eburnation of articular cartilage (arrow) [H&E, 100x]. D- Severe decrease of blue stained in eburnation of articular cartilage (arrow) [Alcian blue stain, 100x].

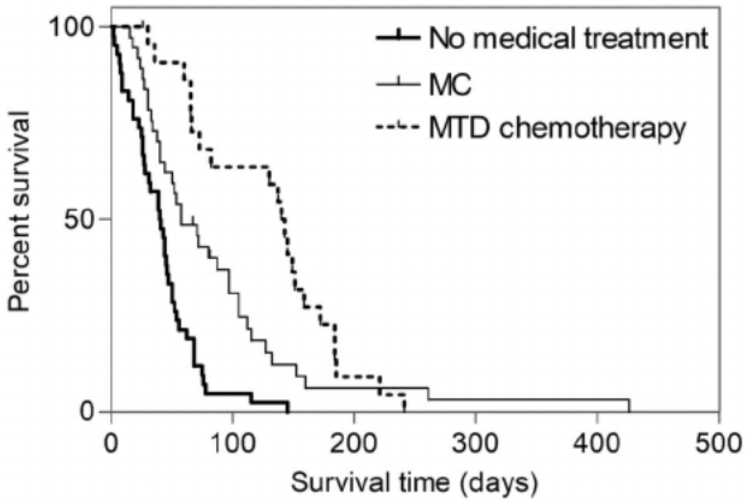
Articular cartilage damage begins with the release of cytokines that triggers the degradation cascade of collagen in the cartilaginous matrix. With the progress of collagen depletion, cartilage loses its ability to absorb impact, and continuous exercise results in constant trauma and consequently cartilage insults. Synovial changes are the result of inflammation including hyperplasia and fibrosis. Bone changes follow in these cases and are characterized by the formation of peri-articular osteophytes, decreased joint space and sclerosis in the subchondral bone [24]. In one study, the process of osteoarthritis was characterized microscopically by the loss of proteoglycans (reduction of metachromatic staining by Toluidine blue) caused by the enzymatic degradation of articular cartilage (mainly by aggrecanase and metalloproteinases). Later on the process, it can be seen fragmented collagen fibers. Exposure and calcification of the cartilaginous layers are also observed, with consequent eburnation of the subchondral bone surface [14]. The Toluidine blue stain is usually used in the histological analysis to demonstrate the presence of glycosaminoglycans in the extracellular cartilaginous matrix, staining in light blue and dark purple (metochromasia) [11]. The samples of the articular cartilage degeneration obtained from the femoral lateral trochlear presented weak marking (light blue) and there was no metachromatic in Toluidine blue staining [23]. Toluidine blue was not performed in this study. Histologic examination in this study of AC by AB stain demonstrates the decrease of blue staining in lesions such as fibrillation of the AC, but the cause of this change was not determined.

a) O trecho retirado do artigo acima faz a descrição histoquímica de lesões osteoarticulares em equinos. Baseado nisso descreva um protocolo de tratamento utilizando terapias convencionais e integrativas, justificando suas escolhas (2,5 pontos).

QUESTÃO 6:

Um médico veterinário recebe um cachorro com hemangiosarcoma esplênico e vai em busca de artigos científicos para embasar sua conduta clínica. Um dos trabalhos que ele encontra é um estudo retrospectivo que compara opções terapêuticas. Alguns resultados são demonstrados abaixo e baseado nesses resultados alguns questionamentos são feitos. *Abreviaturas importantes: MC: Metronomic chemotherapy/ MTD: maximum-tolerated-dose chemotherapy.*

a) **Descreva os resultados apresentados no gráfico abaixo e conclua qual seria a melhor opção terapêutica (0,75 pontos).**



Survival time was significantly longer for dogs treated with MTD than dogs treated with MC (*P* = .023) or splenectomy alone (*P* < .001)

b) **A tabela abaixo demonstra os eventos adversos relacionados a MC e MTD. Comente sobre os resultados apresentados na tabela (0,75 pontos).**

TABLE 3 Adverse events recorded in 61 dogs presenting with metastatic splenic hemangiosarcoma treated with splenectomy plus metronomic chemotherapy or maximum-tolerated dose chemotherapy

	Metronomic chemotherapy (n = 38)	Maximum-tolerated dose chemotherapy (n = 23)	
Number of dogs with adverse events	6 (15.8%)	10 (43.5%)	p=0,017
Adverse events recorded for each dog (number of episodes)	Hematuria grade 1 (1) GI grade 1 (1) Hematuria grade 1 (1) GI grade 1 (1) GI grade 1 (1) GI grade 2 (1)	BM grade 2 (2), GI grade 2 (3) BM grade 2 (2) BM grade 1 (1) BM grade 1 (1) BM grade 4 (1) GI grade 3 (1) BM grade 2 (1) GI grade 1 (1) BM grade 3 (1), BM grade 2 (1) GI grade 3 (2), lethargy grade 1 (1)	
Dose decrease	0 (0%)	4 (17.4%)	
Hospitalization	0 (0%)	5 (21.7%)	p=0,005

Abbreviations: BM, bone marrow; GI, gastrointestinal.

c) Ao ver os resultados acima, qual seria a conclusão do médico veterinário (1,0 ponto)?