JÉSSICA MARCELA VIEIRA PEREIRA

DIGESTIVE ASPECTS OF HOLSTEIN HEIFERS PREGNANTS

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

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“It is far better to launch in search of grandiose achievements,
even exposing themselves to failure, than to align themselves with the poor in spirit,
who neither enjoy much nor suffer much, because they live in a gray gloom, where they
know neither victory nor defeat."

(Theodore Roosevelt)

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BIOGRAPHY

JÉSSICA MARCELA VIEIRA PEREIRA, son of João Peirera de Freitas and Geralda Maria Vieira de Sousa Freitas, was born in Caratinga/MG-Brazil on March 21, 1991.

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On February 22th of 2018, Pereira defended his master’s dissertation to obtain the Magister Scientiae degree in Animal Science.

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ABSTRACT

PEREIRA, Jéssica Marcela Vieira, M.Sc., Universidade Federal de Viçosa, February, 2018. **Digestive aspects of Holstein heifers pregnants** Adviser: Polyana Pizzi Rotta Costa e Silva.

As for the nutrition of pregnant heifers, there is a lack of information on the digestive aspects. This study, for example, for the estimated day of gestation (DG) in Holstein heifers on intake, digestibility, intake, passage and digestion rates, ruminal flow, and blood metabolites of pregnant Holstein heifers. These measures were measured in 12 pregnant Holstein heifers, and of these 8 fistulated in the rumen at 150, 205 and 260 days of gestation (DG). The animals received a diet with corn silage, soybean meal, corn meal, minerals and vitamins in a ratio of bulky: concentrate of 50:50. The GMD during the study was 0.9 kg / day. Total fecal samples were collected to estimate the digestibility of dry matter (DM) and dietary nutrients: organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF), water consumption, digesta omasal for bacterial isolation and ruminal flow estimation. Also, ruminal contents were emptied in order to obtain the rates of passage and digestion of DM and nutrients. Blood collections were collected to analyze metabolites (NEFA, BHB, urea, and glucose). Weights and biometric measurements were performed at the end of each collection period to evaluate the development of the animals. The data were analyzed according to a completely randomized design, using the MIXED procedure of the SAS (SAS, 2008), ruminal and omasal pH measurements were included in the statistical model as time-repeated measures, differences were declared when $P < 0.05$. Higher intakes of DM, OM, CP and NDF (kg / d) were found at 205 compared to 150 DG. In relation to DMI in % BW there was reduction from 205 to 260 DG. For ruminal flow and digestibility, no difference was observed for the parameters of DM, OM, CP and NDF, the same was observed for microbial protein synthesis, digestion parameters and ruminal pool of DM and NDF throughout the gestation periods evaluated. The apparent total digestibility of DM, OM and CP increased from 150 to 205 DG and for NDF no change was observed. The glucose content decreased in the last evaluated period in relation to the first one, there was an increase of beta-hydroxybutyrate and AGNE at the 260 DG when comparing with the 150 DG. Among the measurements performed, only the measurements of height of withers and body length showed a difference between the evaluated periods. Our results demonstrate an increase in the digestibility of DM, OM

and CP from 205 DG and that ki, kp and kd of heifers in gestation do not change during pregnancy. Also, there was a reduction of DMI in relation to CP at the end of gestation as predicted by the NRC (2001) in 14.28%, reaching consumption of 1.47% BW at 260 DG. The values found for blood metabolites do not indicate metabolic diseases. Likewise, the observed pH values are in agreement with the expected after consumption of the diet. Studies evaluating such factors are few in the literature, so more research is needed to consolidate such results.

RESUMO

PEREIRA, Jéssica Marcela Vieira, M.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Aspectos digestivos de novilhas Holandesas gestantes**. Orientadora: Polyana Pizzi Rotta Costa e Silva.

No que se refere a nutrição de novilhas leiteiras em gestação, há falta de informação sobre os aspectos digestivos. Este estudo, portanto, teve por objetivo avaliar o efeito dos dias de gestação (DG) de novilhas Holandesas sobre o consumo, digestibilidade, taxas de ingestão, passagem e digestão, fluxo ruminal de nutrientes, e concentração de metabólitos sanguíneos de novilhas Holandesas gestantes. Tais variáveis foram mensuradas em 8 novilhas fistuladas Holandesas gestantes com peso corporal médio de 450 kg ±27.6 kg, foi utilizado mais 4 novilhas, totalizando 12 animais onde foi mensurado consumo de nutrientes, água e concentração de metabólitos sanguíneos. As mensuração foram realizadas nos 150, 205 e 260 DG. Os animais receberam uma dieta com silagem de milho, farelo de soja, fubá de milho, minerais e vitaminas em uma relação de volumoso:concentrado de 50:50. O ganho médio diário (GMD) durante o estudo foi de 0,9 kg/dia. Foram realizadas coletas totais de fezes para estimar a digestibilidade da matéria seca (MS) e dos constituintes da dieta: matéria orgânica (MO), proteína bruta (PB) e fibra em detergente neutro (FDN). Além disso medição do consumo de água, coleta de digesta omasal para isolamento de bactérias e estimação da digestão ruminal. Ainda foram realizados esvaziamentos do conteúdo ruminal com intuito de se obter as taxas de passagem ingestão e digestão da MS e dos constituintes das dietas. Coletas de sangue foram feitas ao final de cada período de coleta para analisar os seguintes metabólitos: ácido graxo não esterificado (AGNE), beta-hidroxibutirato (BHB), ureia e glicose. Pesagens e medidas biométricas foram realizadas ao final de cada período de coleta para avaliar desenvolvimento dos animais. Maiores consumos de MS, MO, PB e FDN (kg/dia) foram encontrados aos 205 em relação aos 150 DG. Em relação ao consumo de matéria seca (CMS) em porcentagem do peso corporal (%PC) houve redução dos 205 para 260 DG. Para o fluxo ruminal e digestibilidade ruminal não ocorreu diferença para os parâmetros de MS, MO, PB e FDN. Mesmo comportamento foi observado para síntese de proteína microbiana, parâmetros de digestão e pool ruminal da MS e FDN ao longo dos períodos de gestação avaliados. A digestibilidade total aparente da MS, MO e PB aumentou dos 150 para 205 DG e para FDN não foi observada alteração. O teor de glicose diminuiu no último período avaliado em relação ao primeiro; e ocorreu aumento de beta-hidroxibutirato e

AGNE aos 260 DG quando comparado aos 150 DG. Dentre as medições realizadas, somente as medidas de altura de cernelha e comprimento corporal apresentaram diferença entre os períodos avaliados. Estes resultados demonstram que a partir dos 205 DG de novilhas ocorre aumento da digestibilidade total para MS, MO e PB. Para as taxas ki, kp e kd não ocorre alteração ao longo da gestação. Redução do CMS em relação ao PC ao final da gestação é observado como previsto pelo NRC (2001) em 14,28% atingindo consumo de 1.47% do PC aos 260 DG. Estudos avaliando tais fatores são poucos na literatura, assim mais pesquisas são necessárias para consolidar os resultados encontrados.

INTRODUCTION

According to NRC (2001), the recommended body weight for dairy heifers at first calf is 82% of mature BW. Thus, their nutritional plan requires strategies to maintain constant and satisfactory gains until the end of gestation (Heinrichs et al., 2017). According to Mohd et al. (2013), the advances in reproduction were superior to the nutritional advances of heifers, producing precocious animals that may present low milk yield in the first lactation. To increase the efficiency of these heifers, new approaches must be adopted, and for this reason, it is necessary to find an accurate method to gather relevant information that will improve the nutritional performance during gestation.

Regarding the nutrition of dairy heifers, the literature lacks information on the digestive aspects in relation to ruminal flow, ingestion, passage and digestion rates, during gestation of heifers, what is found in the literature are studies that address nutrient fluxes of non-pregnant, lactanting animals (Doorenbos et al., 2016; Ramirez et al., 2016; Berends et al., 2015) or when in gestation studies in the peripartum with cows (Stanley et al., 1993), are also mathematical models that were developed from data obtained from in vitro and in situ techniques to increase the efficiency of feed use and to estimate values for rates of passage, digestion,), and determining the outflow of rumen digesta (Allen and Linton, 2007; Gregorini et al., 2015; Banninck et al., 2016).

Data regarding nutrition of dairy cattle during gestation in the literature are focused on dairy cows and are scarce for heifers (NRC, 2001; Heinrichs, 2017; Akers, 2017). It is well known that dry matter intake (DMI) decreases during the final third of gestation due to the greater size of the gravid uterus and the increased estrogen concentration (Bertoni and Trevisi, 2013; Rotta et al., 2015a) in cows. This decrease in DMI may reach 50% in the last two months of gestation in dairy cows (Rotta et al., 2015a). However, it is not known whether the DMI in heifers has a similar decrease of this magnitude since they are still growing; such information would be of great use to improve management practices in pregnant heifers.

The hypothesis of this study was that the digestive parameters of pregnant heifers would be different from the data in the literature for pregnant cows and would also be altered during pregnancy. Thus, the objectives of this study were to estimate ruminal flow, total and partial digestibility of dry matter and diet constituents (total), intake, passage and digestion rates, water consumption, blood metabolite concentrations and measures body during gestation in Holstein heifers.

MATERIAL AND METHODS

All procedures that were implemented in this study were previously approved by the Animal Ethics and Welfare Committee of the Federal University of Viçosa according to protocol number 020-2016.

Experimental Design

Twelve pregnant Holstein heifers, eight of which fitted with a rumen cannula, with a mean body weight of 450 kg ± 27.6 kg and mean initial age of 20 ± 3.5 months were used in the experiment. The treatments were the following days of gestation (DG): 150DG, 205DG and 260DG. The animals were allocated to individual stalls of 10 m². Prior to the start of the experiment, heifers underwent a 30-day adaptation period to experimental facilities and conditions. Heifers received the same diet (Table 1), for an ADG of 0.9 ± 0.15 kg/day, according to NRC, 2001.

The roughage:concentrate ratio was 50:50 (DM basis) and was delivered daily at 7h00 a.m. and 3h00 p.m.. Animals were allowed 5% of leftovers. Once a week, corn silage was sampled and dried in a ventilated oven at 55ºC for 72 hours to correct the DM content.

The collection periods were established according to the days of gestation: 145-154, 200-209, and 255-264, with a duration of ten days for each period (Figure 1).

Intake and digestibility trial

Dry matter intake was measured daily by weighing offered and refused feedstuffs. Water intake was also measured for 6 consecutive days during each period (first period: 145-150 DG, second period: 200-205 DG and third period 255-260 DG). At the end of 24 hours, the volume of water in each vessel was measured and filled again with a known volume.

To estimate the digestibility of nutrients, total feces were collected from all heifers for 3 consecutive days in first period: 145-150 DG, second period: 200-205 DG and third period 255-260 DG. After 24 hours of collection, the total feces were weighed and approximately 250 g was dried in a ventilation oven (55ºC for 72 hours). After each collection period, a composite sample was created referring to the 3 days of fecal collection, based on DM and fecal production. Then the feces and feed samples were milled with 1 and 2 mm sieve knives and stored for further analysis.

Collection of omasal and ruminal digesta and ruminal emptying

A continuous infusion of 5 g/d (0.7 g cobalt) of Co-EDTA from the 1st to the 6th day of collection was performed during each period (first period: 145-150 DG, second period: 200-205 DG and third period 255-260 DG) by using 2 peristaltic pumps (model BP-600.4, Colombo, Paraná, Brazil). A total of 8 samples were collected from the omasum at a 9-hour interval for 3 days; sampling times were 02:00, 05:00, 08:00, 11:00, 14:00, 17:00, 20:00, and 23:00 hours. The technique that was developed by Huhtanen et al. (1997) and adapted by Leão (2002) was used for sampling of the omasal digesta, where approximately 250 mL of digesta was used for a bacterial isolate and 500 mL was used for the ruminal flow estimation (Rotta et al., 2014a). The samples were stored in plastic pots at -20 °C for further analysis.

At the end of each experimental period, samples of omasal digesta were thawed at room temperature and one composite was created per animal following the recommendations of Rotta et al. (2014a), thus obtaining approximately two liters of digesta, which were destined to the isolation of bacteria. The isolation of bacteria was performed according to Reynal et al. (2005), with modifications suggested by Krizsan et al. (2010). The bacterial samples were divided into liquid-associated bacteria (BAL) and bacteria associated with particles (BAP). Bacterial samples were stored at -80° C and then lyophilized. Four liters were obtained to estimate the omasal flow, which was filtered by using a 100 μm nylon filter with an open area of 44% (Sefar Nytex 100/44; Sefar, Thal, Switzerland), thereby obtaining two phases. Thus, the double indicator system technique was used to estimate the omasal flow of digesta during the gestation period. All samples were stored in plastic containers and kept at -80° C for further lyophilization. After lyophilization, samples were milled in 2 and 1 mm knife mills.

Ruminal contents were sampled during the sampling period and were collected at all times of omasal collection, totalizing 9 samplings. Samples were manually collected at the liquid-solid interface of the rumen, filtered through a 100 μm nylon filter, and were subjected to pH measurements in addition to the omasal digesta samples by using a digital potentiometer (Ph-221, Lutron Electronics, Taiwan).

On the 7th day of collection for each experimental period, total rumen emptying was carried out 4 hours after feeding to estimate the rate of passage and digestion (Allen & Linton, 2007). After removal of all ruminal contents, the digesta was weighed and filtered for separation of solid and liquid fractions, which were then weighed and sampled. After sampling, the digesta was reconstituted and returned to the rumen of the respective animals. On the 9th day, the entire rumen emptying procedure was repeated, this time before the morning feeding. The collected samples were lyophilized and ground in a knife mill with 1 mm sieves. Thus, composite samples of the dried solid and liquid fractions after emptying the rumens were obtained and were based on the dry weight of each sample.

Blood measurements

Blood samples were collected via jugular venipuntucture by using vacuum tubes with clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil) in order to quantity urea, non-esterified fatty acids (NEFA), and beta-hydroxybutyrate (βHB); an extra tube with EDTA and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) was used to quantity plasma concentration of glucose. After collection, samples were centrifuged at 3600 × g for 20 min, and serum and plasma were immediately frozen at -20°C until further analysis.

Bioclin® kits were used to quantity urea (K056) and glucose (K082). NEFA and βHB were analyzed by using Randox® kits (FA115 and RB1007, Antrim, United Kingdom). All mentioned analyses were determined by an automated biochemical analyzer (Mindray, BS200E, Shenzhen, China).

Biometric Measurement and Weighing

At the end of each experimental period (first period: 154 DG, second period: 209 DG and third period 264 DG), the animals were fasted for 12 hours and were then weighed. In sequence, length of teats, withers height (WH), croup height (CH), croup width (CW), body length (BL), and thoracic perimeter (TP) were measured while the animals were kept in a standing position within shuttle; WH, CH, CW, and BL were measured using a hipometer and TP was measured with a measurement tape.

Laboratory analysis

Samples of omasal digesta, feces, feed, and ruminal emptying were analyzed for DM, organic matter (OM), and nitrogen (N) (AOAC, 2000 - method 934.01 for DM, 930.05 for OM, and 981.10 for N, respectively). The ether extract analysis was performed according to AOAC recommendations (2006, method 945.16). The neutral detergent fiber corrected for ash (NDF) was analyzed according to the technique described by Mertens (2002) without the addition of sodium sulphite, but with the addition of detergent thermostable alpha amylase (Ankon Tech Corporation Fairport, NY). Ash correction was performed in the NDF residues (INCT-CA M-002/1). Indigestible NDF (iNDF) was estimated in triplicate for omasal digesta (particle phase), fecal samples, and rumen emptying samples. Non-fibrous carbohydrates were calculated according to Detman & Valadares Filho (2010), with no correction for urea.

The samples of omasal digesta were analyzed by an atomic absorption spectrophotometer (SPCTR AA-800; Varian spectrometer, Harbor City, CA) according to the method described by Kimura & Miller (1957). The BAL and BAP were analyzed for purine bases as recommended by Ushida et al. (1985), with modifications by Zinn & Owens (1986). Furthermore, BAL and BAP samples were analyzed for DM, OM, and CP.

Calculation

The total digestibility was calculated by the difference between consumed and excreted. Ruminal digestibility coefficients were estimated by measuring the difference between the rate of nutrient intake and the flow of the nutrients through the rumen. The calculation of intestinal digestibility was estimated by determining the concentration of nutrients in omasal digesta and feces. The flow of the omasal digesta was estimated through the reconstitution of digesta technique that was developed by Faichney (1975) using the double marker system. The use of the double marker system is well established and its use has been extensively documented in several studies (Faichney 1975; France & Siddons, 1986; Rotta et al., 2014). Cobalt was used as the liquid phase marker and the iNDF as the particle phase marker. The reconstitution factor was calculated based on the concentrations of the markers during the different phases of the digesta (France & Siddons, 1986).

The rates of ingestion (k_i), passage (k_p), and digestion (k_d) were calculated by using the pool-and-flux method described by Allen & Linton (2007), according to the following models:

$$ki = \left(\frac{ingestion}{rumenpool}\right) * 100$$

$$kp = \frac{rumenflow}{rumenpool}$$

$$kd = \left(\frac{ingestion}{rumenpool}\right) - Kp$$

where:

k_i = ingestion rate of feed fractions (% h^{-1});

intake = feed intake (kg h^{-1});

rumen pool = amount of total rumen dry matter (kg);

k_p = passage rate of feed fractions (% h^{-1});

rumen flow = amount of dry matter or nutrients in the omasum (kg h^{-1});

k_d = digestion rate of diet fractions (%h^{-1}).

Statistical analysis

Data were analyzed according to a completely randomized design using the SAS MIXED procedure (SAS, 2008), according to the statistical model below:

$$Y_{ij} = \mu + DG_i + \varepsilon_{ij} \ ,$$

where Yij = dependent variable, μ = general constant, DGi = fixed effect of days of gestation, and □ij = random error.

Ruminal and omasal pH measurements were included in the statistical model as time-repeated measures (time effect). The fixed time effects and the interaction between time and treatment were tested by using the appropriate (co) variance structure. The following (co) variance matrices were tested: components of variance, composite symmetry, heterogeneous composite symmetry, and first order heterogeneous auto-regression. The selection of the (co) variance matrix was based on the Akaike-corrected criterion, and the composite symmetry matrix was chosen. For all analyses, multiple comparisons between treatments were performed using the "t" test and differences were declared when $P < 0.05$.

RESULTS

Dry matter intake, nutrients, ruminal flow, and digestibility

Free water intake in liters per day (L/day) did not differ ($P = 0.2025$) between the 3 gestation times that were evaluated (Figure 4A). Intake of water in L/d showed an average of 29.2 L / d for 150 DG, 38.2 L/d for 205 DG, and 33.3 L/d for 260 DG. Regarding the water intake in relation to the BW (Figure 4B), there was no difference ($P = 0.195$) between the days of gestation, with a mean of 6.16 L/d/BW.

The animals increase ($P = 0.03$) DMI, expressed in kilograms per day (kg / d), at 150 DG compared to 205 DG (Table 2). As for nutrient intake, the same behavior was observed, with an increase of 13.02% ($P = 0.03$) for OM, 28.45% ($P < 0.01$) for CP, and 18.00% ($P = 0.04$) for NDF at the end of gestation (260 DG). Regarding DMI in % CP according to the gestation times (Figure 2), it was observed that in the first evaluated period (150 DG) there was no reduction in relation to the 205 DGs. However, a reduction ($P < 0.01$) in DMI was observed at 205 (1.68% BW) to 260 (1.47% BW) DG of 14.28%. Regarding the ruminal flow, there was no difference for the parameters that were evaluated: DM ($P = 0.29$), OM ($P = 0.27$), CP ($P = 0.26$), and NDF ($P = 0.06$, Table 2). The same was observed for ruminal digestibility of DM ($P = 0.36$), OM ($P = 0.34$), CP ($P = 0.09$), and NDF ($P = 0.46$). In addition, for the microbial protein synthesis, no difference ($P = 0.19$) was observed between the gestation periods that were evaluated.

Digestion kineticscts

The values for ki, kd, and kp of DM at different times of gestation did not differ ($P > 0.10$, Table 3). The means for ki, kd, and kp of DM was 7.36 ± 0.456, 2.97 ± 0.300, and 5.17 ± 0.740, respectively. Meanwhile, for NDF the mean ki was 5.23 ± 0.800, kd was 2.84 ± 0.283, and kp was 2.39 ± 0.320.

There was no difference ($P > 0.10$) for the ruminal pool of DM and NDF between the DGs that were studied, thereby presenting with an average ruminal pool of 4.8 ± 0.21 kg for DM and 1.31 ± 0.090 kg for NDF throughout the evaluated gestation times.

Apparent total-tract digestibility

The apparent total-tract digestibility of DM, OM, and CP was altered ($P < 0.01$) by DG, with an increase of 10.8, 8.5, and 9.8%, respectively, when comparing the 205 and 150 DG (Figure 3 A, B, and C). For the NDF (Figure 3D), there was no difference ($P = 0.194$).

Blood metabolites

The urea concentration did not differ ($P = 0.4921$) between the evaluated times. Glucose levels decrease ($P < 0.01$) by approximately 7.18% from 150 DG to 260 DG. On the other hand, levels of βHB ($P < 0.0252$) and NEFA ($P < 0.0222$) increased from 150 DG to 260 DG.

Ruminal and omasal pH

Values for pH differed during the 3 evaluated gestation times for ruminal fluid ($P < 0.0001$) and for omasal fluid ($P = 0.0472$) (Figure 4). The interaction effect between the treatments and these hours was obtained for omasal digesta at 5h ($P < 0.0001$), 8h ($P = 0.0247$), 11h ($P = 0.047$), and 14h ($P = 0.0431$). In relation to ruminal fluid, the interaction effect was present at 5h (P = 0.0002), 17h (P = 0.0123), and 23h (P = 0.0033).

Body measurements

Among the measurements TH, SH, CH, BL, and TP, only the measurements of SH (P = 0.0264) and BL (P < 0.0001) presented with a difference between the periods that were evaluated (Table 5). The measurements of the teats were not diferent between the evaluated periods; however, it was observed that the anterior teats were 16.6% larger than the posterior teats (Table 5).

DISCUSSION

Dry matter intake and nutrients, ruminal outflow, and digestibility

NRC (2001) reported that the DMI of growing heifers during the last trimester of gestation is non-existent. In this study, when comparing DMI at the 3 gestation times that were analyzed, it was observed that at the 150 DG in relation to the 205 DG, there was an increase of approximately 13% in the consumption expressed in kg/d. This can be explained by the fact that heifers are still growing and have an ADG around 0.9 kg/d, which is not the case with adult cows who have a reduced DMI during pregnancy (Bertoni and Trevisi, 2013). Similarly, nutrient intake also showed the same behavior as DM in this study.

However, it was observed that DMI expressed in %BW was constant at 150 DG and 205 DG and suffered a 15% decrease at 260 DG, which corresponds to the 37th week of gestation, approximately. This reduction was also reported by Linden et al. (2008), Park et al. (2011), and Rotta et al. (2015). The reduction of DMI can be influenced by multiple factors (Baile and Mc Laughlin, 1987) like an increase or decrease in the concentration of hormones such as estrogen (Teixeira et al., 2003), as well as the reduction of rumen physical space at the end of gestation (Rotta et al., 2015), which, according to Forbes (1968), is the most limiting factor of consumption.

An animal during the period of late-gestation presents with high energy and protein demands due to colostrum production, final fetal growth, and maintenance of the cow itself (Overton, 2013). In the case of heifers, energy and protein are still required for their growth (NRC, 2001). Thus, if heifers have large reductions in DMI, greater consequences will be observed in the postpartum period, such as a high loss of BCS, delay in postpartum ovulation, ketosis, and others, with a greater proportion of consequences for cows of high production (Butler, 2005).

In their study, Brscic et al. (2015) evaluated metabolite values (albumin, urea, triglycerides, fatty acids, among others) as a reference for heifers during late-gestation, while comparing the values for primiparous and multiparous cows. They observed higher levels of FA with an increase in the number of deliveries, and as a result, the authors of the current study justified this increase due to the reduction of DMI, as reported by Chapinal et al. (2010) and Ospina et al. (2010), thereby resulting in mobilization of adipose tissue and consequently higher concentrations of plasma FA. In this study, the DMI was constant, just like the BCS, which could lead to a severe postpartum NEB.

Digestion kinetics

In the hypothesis of this study, it was expected that the evaluated parameters ki, kp, and kd would present with changes at the end of gestation. An example would be the rate of passage, which was expected to increase at the end of gestation, as observed by Weston (1979), Stanley et al. (1993), and Reynolds et al. (2004) due to the increase in DMI, which would therefore decrease nutrient digestibility (Tyrrell and Moe, 1975). However, the same pattern was not observed in this study; the rates remained constant, with no differences in the gestation times that were evaluated. As for kd, Reynolds et al.

(2004) observed an increase in rates over the trimesters of gestation, and this result was not expected; given the increasing effect of kp, this result would not be expected for kd, but there is also a possibility that rumen elasticity allowed room for increased intake (Forbes, 1968).

Park et al. (2011) point out that a number of factors, such as physiological, gastrointestinal, and food changes, should be considered to explain response rates in addition to food characteristics such as particle size. Thus, these factors may have influenced the lack of difference along the DG when these variables were assessed.

Seo et al. (2006) report that in order to make predictions of kp with greater precision and to better explain the physical properties of the particles, the liquid flow and the variation of the intake that affect the rate of passage are necessary to better establish an equation model for kp. As further explained by Seo et al. (2006), the model for NRC protein (2001) is used to update feed formulation programs for dairy cows in order to improve accuracy in predicting protein and dietary amino acids and to reduce N excretion and feed cost.

Further studies should be performed to better elucidate rumen parameters at late-gestation in Holstein heifers, so that they serve as the basis for formulating rations to meet the requirements of this category, since the focus in the studies are based on lactating cows.

Apparent total-tract digestibility

As a consequence of the lack of change in the passage rate, the digestibility also did not present with a reduction (Figure 3). McCarthy et al. (1988) evaluated lactating Holstein cows and found a higher rate of passage of corn based diet constituents, which yielded an increased intake and thus decreased digestibility. Increased digestibility was observed for MS, MO, and PB (Figure 3)for Park et al. (2011) when evaluating the ruminal dynamics of pregnant cows during the peripartum period.

The study by Sousa et al. (2017) when evaluating the digestibility of nutrients in dairy cows (1,942 observations - from 54 studies) found an inverse relationship between DMI in %CP and its digestibility. The same behavior was verified in this study, with a reduction of DMI %BW of 14.23% and an increase in DM digestibility of 10.8%. This result implies a greater use of food by the animal, even when it presents with a reduction in consumption, as observed in this case in relation to the BW.

The NRC (2001) reports use only low consumption cows to estimate digestibility, so a better understanding of the digestibility behavior of different diets with other categories of animals (eg, heifers, high-lactating cows) is necessary in order to more accurately estimate the digestibility of other animals, according to Souza et al. (2017).

Free water intake

Water, in addition to its fundamental importance in the regulation of physiological functions in the body (Squires, 1988) and in milk production, where it comprises 87% of its composition (Oliveira et al., 2010), is the most important nutrient for cattle milk (NRC, 2001).

The increased water consumption affects the CMS positively and can also be influenced by the ratio of the temperature-humidity index of the environment.. According to Baumgard and Rhoads (2012), indices greater than 68 result in animals with signs of thermal stress. In the present study, the temperature index of average humidity throughout the experiment was 65.53 ± 3.47, and was thus below the index of thermal stress.

Water consumption averaged 33.6 L/d over the three periods. Several studies have developed equations to predict this consumption according to the CMS (Litle and Shaw, 1978; Murphy et al., 1983; Holter and Urbar, 1992). The NRC (2001) recommends the use of the equation proposed by Murphy et al. (1978) for water consumption in lactating cows, which considers DMI, milk production, sodium consumption, and environmental temperature. Mathematical models were proposed to predict water intake by analyzing 55 studies, and the following equation was proposed for dry cow consumption: FWI (kg / d) = $1.16 \pm 0.38 \times$ DMI + $0.23 \pm 0.08 \times$ DM% + $0.44 \pm 0.16 \times$ TM + $0.061 \times$ TMPC. When using the data from this study and the equation, water consumption presented with an average of 31.467 kg/d, which was 6.65% lower than the one that was measured.

Blood metabolites

Several factors affect the concentration of metabolites in the blood of lactating cows (age, physiological state, among others) (Shaffer et al., 1981). A number of studies using lactating cows have been carried out and reference limits of several metabolites have been established (Ospina et al., 2010, Cozzi et al., 2011, Overton et al., 2017). However, according to Brscic et al. (2015), reference values for heifers at the end of gestation have not been studied.

In this study, values for glucose and urea were similar to those found by Brscic et al. (2015): minimum concentration of 2.8 mmol / L and maximum of 4 mmol / L for glucose, and for urea the values were between 1.1 mmol / L and 5.8 mmol / L, thus establishing such values as a reference for heifers during late-gestation.

For βHB, values reached levels of 0.44 ± 0.044 mmol / L at 260 DG. According to Duffiel et al. (2009), the blood concentration of βHB is utilized for the diagnosis of ketosis, a disease that is associated with a negative energy balance and which is diagnosed when the values reach concentrations higher than 1.2 mmol / L, which is 3 times higher than values in this study.

The values that were found for NEFA reached 0.34 ± 0.054 mmol / L. According to Overton et al. (2017), levels above 0.5 mmol / L in the prepartum period indicate a higher probability of developing some metabolic disease. Thus, heifers with an ADG feeding management of 0.9 kg with the diet that was used in the present study are unlikely to present with metabolic problems due to the low levels of βHB and NEFA.

Ruminal and omasal pH

Ruminal pH can be influenced by diet and feed management (Grant and Mertens, 1992; Owens, 1998); according to Rskov (1986), ruminal pH reduction occurs between 0.5 - 4 hours after feeding. This reduction is due to the digestion of food. It is observed that from 1 to 2 hours after supplying the diet (7 hours and 15 hours) the ruminal fluid pH presented with a reduction, reaching 5.76 4 hours after the diet in the morning and 5.56 in the afternoon 2 hours after the diet delivery during the first gestation period.

The pH of the omasal fluid presented with a similar pattern as the ruminal pH, but it demonstrated a smaller variation during the 3 gestation periods, with a range between 6.04 and 6.88.

According to Van Soest (1994), ruminal pH should be between 6.7 ± 0.5 to maintain an appropriate environment for the activity of the microorganisms that are present in the rumen. According to Berchielli et al. (2011) and Klein (2014), the ideal pH range for microbial growth is between 5.5 - 7, so the values that were found in this study fall within this range.

Body measurements

Body growth measurements are essential indicators for a better evaluation of animal development, and are a consequence of the efficiency of the management, food, sanitation, and labor (Hoffman et al., 1992).

Monitoring these measurements help to ensure an adequate weight of 82% at maturity (NRC, 2001) and also a SH of 1.4 m (Hoffman et al., 1992). In the present study, the results for SH are consistent with that proposed by Hoffman et al. (1992) at the time of calving.

Hazel et al. (2014) evaluated anterior teat lengths (FTL) and TP over 5 lactations in Holstein cows and observed FTL of 4.9 cm and TP of 1.97 m during the evaluated lactations. Heins et al. (2008), when evaluating the first lactation of Holstein cows, found a mean for FTL of 4.4 cm and for TP of 1.9 m. In this study, we observed that the FTL at the end of the 260 DG was 4.14 ± 0.214 cm, which will increase over the future lactations.

In relation to TP, the present study presented with higher values at the end of gestation (2.13 ± 0.071 m) when compared to the average that was obtained by Heins et al. (2008) of 1.89 m and Hazel et al. (2014) of 1.97 m when evaluating this characteristic in lactating animals. A possible justification for this would be that the animals that were evaluated in this experiment had a higher ECC (3.87 ± 0.16) in relation to the animals of the cited studies, which were 2.87 (Hazel et al., 2014) and 2.7 (Heins et al., 2008) respectively.

CONCLUSION

This study presented consumption characteristics, digestive and physiological parameters of pregnant Holstein heifers at 150, 205, and 260 DG. Our results demonstrate an increase in the digestibility of DM, OM, and CP from 205 DG and that ki, kp, and kd of heifers do not change during pregnancy. Also, there was a reduction of DMI in relation to CP at the end of gestation by 14.28%, reaching 1.47% PC at 260 DG. Studies evaluating such factors are scarce in the literature, so further research is still needed to consolidate such results.

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TABLES AND FIGURES

Tabela 1. Ingredientes e composição química dos alimentos

Item	% of DM
Ingredients	
Corn silage	50.0
Soybean meal	25.0
Ground corn	21.6
Limestone	1.07
Sodium bicarbonate	0.75
Magnesium oxide	0.25
Salt	0.50
Vitamins	0.76
Mineral mix[1]	0.02
Chemical composition	
DM	56.3
OM	95.2
CP	17.6
NDF	32.2
Ether extract	2.6
NFC	45.2
Indigestible NDF	8.75

[1]Mineral mix composition = 0.83 mg/kg of cobalt sulfate, 38.6 mg/kg of copper sulphate, 0.215 g/kg iron sulphate, 2,39 mg/kg of sodium selenite, 0.167 g/kg of zinc sulfate, 1.71 mg/kg potassium iodate.

Table 2. Intake, ruminal outflow, ruminal digestibility and production protein microbian in Holstein heifers in different days of gestation

	Days of gestation			
Item	150	205	260	P-value
Intake, kg/day				
DM	7.83[b] ± 0.691	8.97[a] ± 0.691	8.84[a] ± 0.707	0.03
OM	7.45[b] ± 0.668	8.57[a] ± 0.668	8.42[a] ± 0.683	0.03
CP	1.23[b] ± 0.115	1.67[a] ± 0.115	1.58[a] ± 0.120	<0.01
NDF	1.49[b] ± 0.131	1.72[a] ± 0.131	1.76[a] ± 0.143	0.04
Ruminal outflow, kg/day				
DM	4.95 ± 0.697	5.45 ± 0.697	5.28 ± 0.709	0.29
OM	4.86 ± 0.591	5.28 ± 0.591	5.13 ± 0.600	0.27
CP	1.23 ± 0.165	1.33 ± 0.165	1.40 ± 0.170	0.26
NDF	0.67 ± 0.089	0.81 ± 0.089	0.79 ± 0.095	0.06
Ruminal digestibility, %				
DM	37.95 ± 2.392	41.71 ± 2.392	42.88 ± 2.763	0.36
OM	43.71 ± 2.903	48.66 ± 2.903	49.70 ± 3.352	0.34
CP	0.11 ± 8.569	20.98 ± 8.569	11.38 ± 9.315	0.09
NDF	44.99 ± 9.991	47.04 ± 9.991	44.81 ± 11.124	0.46
Microbial protein, g/Kg TDN	123.51[a] ± 11.637	71.87[a] ± 11.637	109.54[b] ± 13.437	<0.01

[a b] Means within a row with different superscript letters differ (Student T test, P <0.05).

Table 3. Digestion kinetics for Holstein heifers in different days of gestation

Item		Days of gestation			P-value
		150	205	260	
Rates, $\%h^{-1}$					
[1]DM	ki	6.91 ± 0.701	7.78 ± 0.701	7.41 ± 0.855	0.10
	kp	4.23 ± 0.786	4.51 ± 0.786	4.45 ± 0.828	0.53
	kd	2.68[b] ± 0.203	3.27[a] ± 0.203	2.96[ab] ± 0.234	0.08
NDF	ki	4.43 ± 0.635	5.84 ± 0.635	5.44 ± 0.734	0.12
	kd	2.41 ± 0.620	3.13 ± 0.620	3.00 ± 0.716	0.10
	kp	2.02 ± 0.372	2.71 ± 0.372	2.44 ± 0.430	0.34
Rumen pool sizes, kg of					
DM		4.88 ± 0.291	4.95 ± 0.291	4.63 ± 0.336	0.76
NDF		1.37 ± 0.139	1.22 ± 0.139	1.34 ± 0.160	0.72

[a b] Means within a row with different superscript letters differ (Student T test, P <0.05).

Table 4. Metabolic parameters of blood samples in pregnant Holstein heifers.

Item	Days of gestation			P-value
	150	205	260	
Glucose (mmol/L),	$4{,}04^{a} \pm 0{,}055$	$3{,}89^{ab} \pm 0{,}055$	$3{,}75^{b} \pm 0{,}064$	0,0085
Urea (mmol/L),	4,96 ± 0,348	5,54 ± 0,348	5,38 ± 0,408	0,4921
β-hidroxibutyrate (mmol/L),	$0{,}28^{b} \pm 0{,}037$	$0{,}37^{ab} \pm 0{,}037$	$0{,}44^{a} \pm 0{,}044$	0,0252
NEFA (mmol/L),	$0{,}19^{b} \pm 0{,}037$	$0{,}16^{b} \pm 0{,}037$	$0{,}34^{a} \pm 0{,}054$	0,0222

[a b] Means within a row with different superscript letters differ (Student T test, P <0.05).

Table 5. Biometric measurements in pregnant Holstein heifers.

Item	Days of gestation			P-value
	150	205	260	
Teat				
Previous right, cm	3,65 ± 0,193	3,94 ± 0,203	4,14 ± 0,214	0,2468
Previous left, cm	3,75 ± 0,219	3,91 ± 0,227	4,01 ± 0,236	0,6255
Posterior right, cm	3,13 ± 0,291	3,15 ± 0,294	3,30 ± 0,298	0,6997
Posterior left, cm	3,04 ± 0,198	3,40 ± 0,204	3,50 ± 0.222	0,1688
Height of withers, m	1,36 ± 0,016	1,39 ± 0,017	1,43 ± 0,019	0,0264
Croup height, m	1,41 ± 0,019	1,45 ± 0,021	1,49 ± 0,022	0,0657
Body length, m	1,21b ± 0,038	1,49a ± 0,041	1,49a ± 0,045	<0,0001
Thoracic perimeter, m	1,89 ± 0,06	2,04 ± 0,063	2,13 ± 0,071	0,0539

[a b] Means within a row with different superscript letters differ (Student T test, P <0.05).

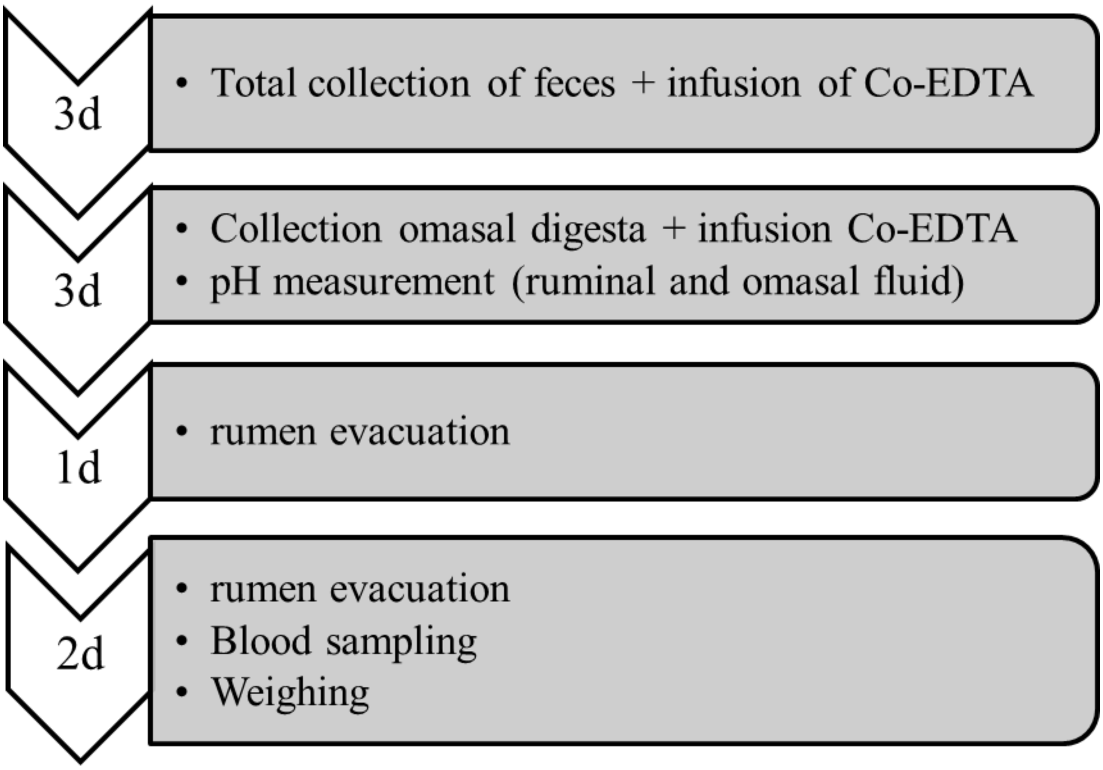

Figure 1. Schematic representation of collection periods.

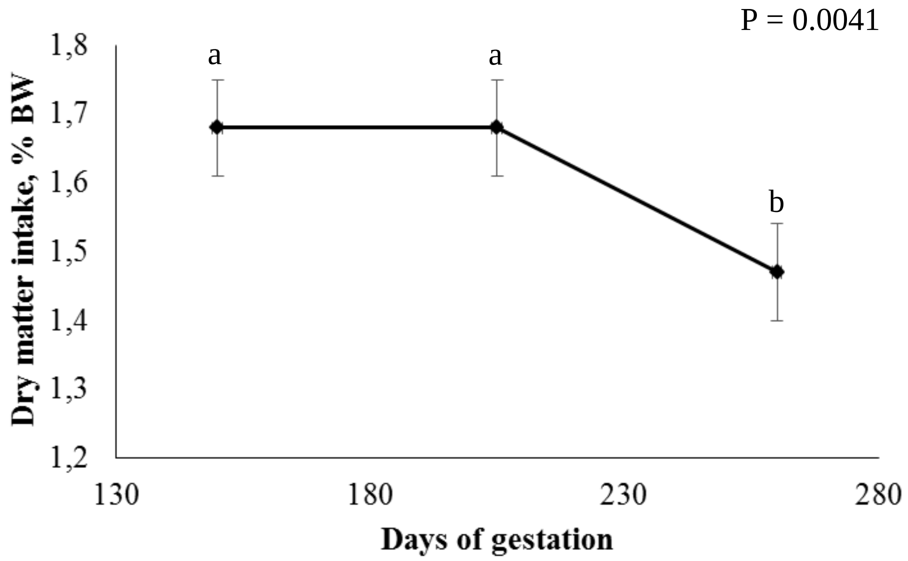

Figure 1. Dry matter intake (% BW) for different gestation times of Holstein heifers.

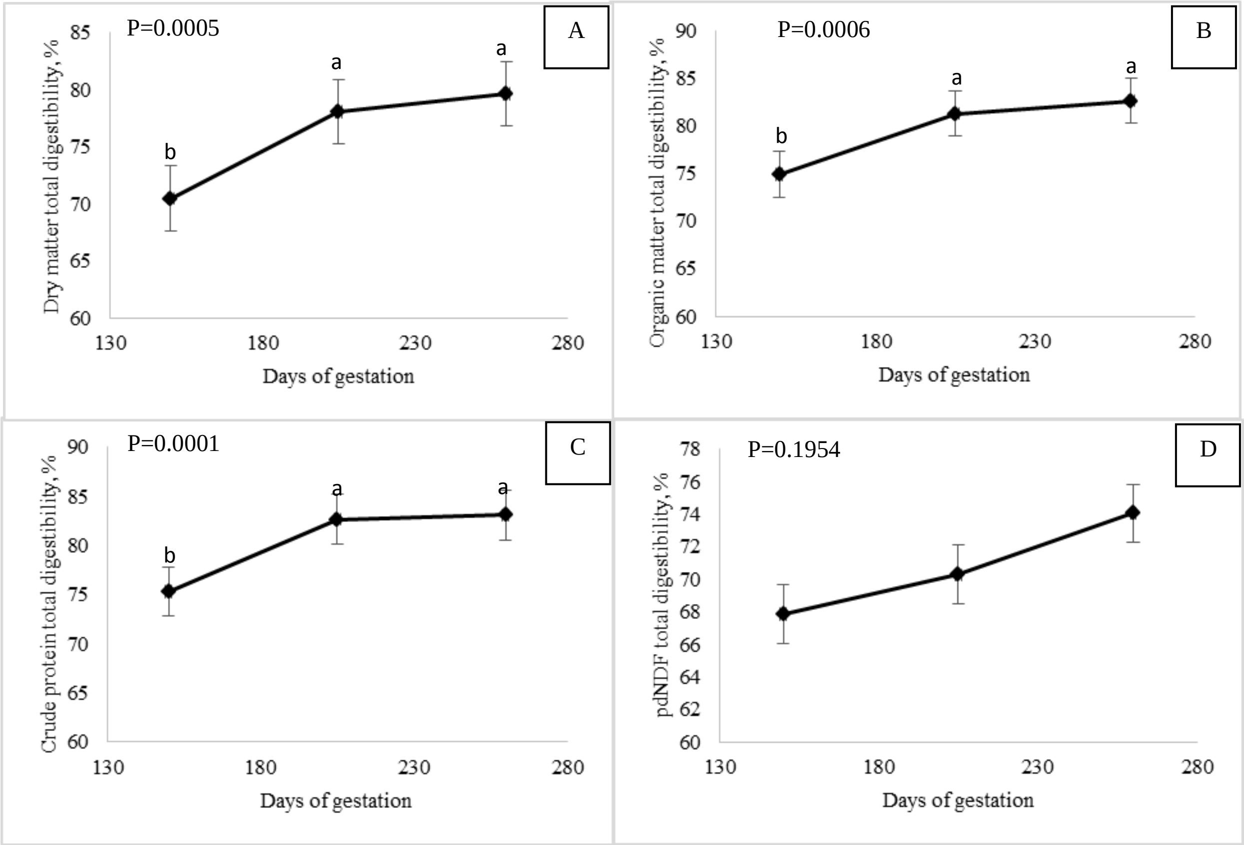

Figure 3 – Apparent total-tract digestibility for different days of gestation in Holstein heifers.

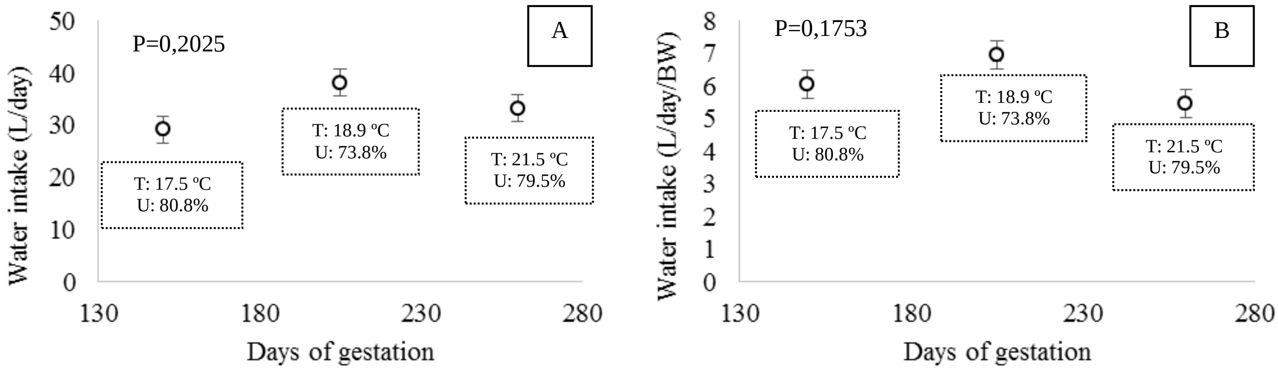

Figure 4. Water consumption for different gestation times of Holstein heifers: A) L / day, and B) L / day / PC.

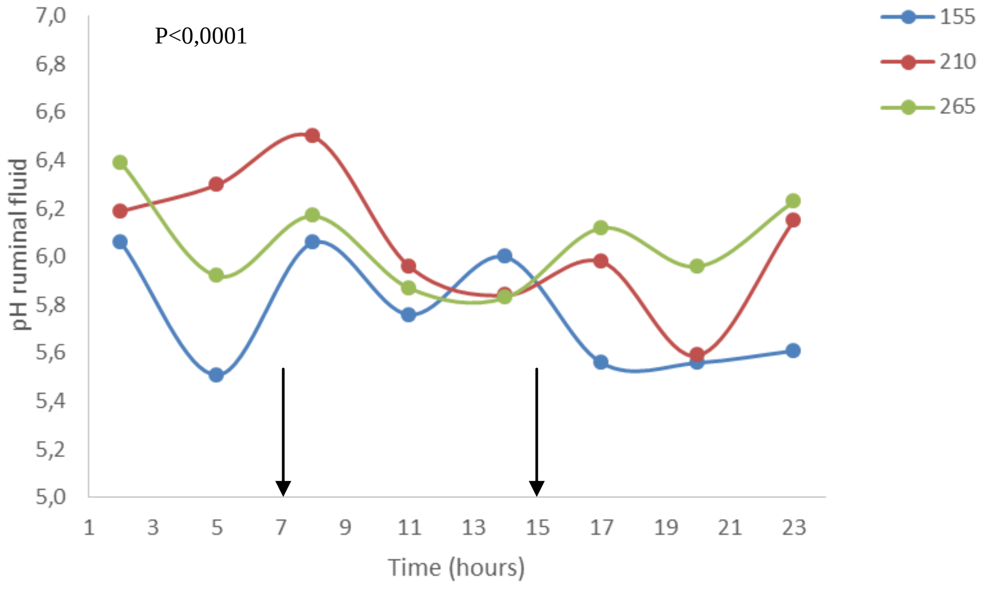

Figure 5. pH of ruminal fluid for different gestation times of Holstein heifers.

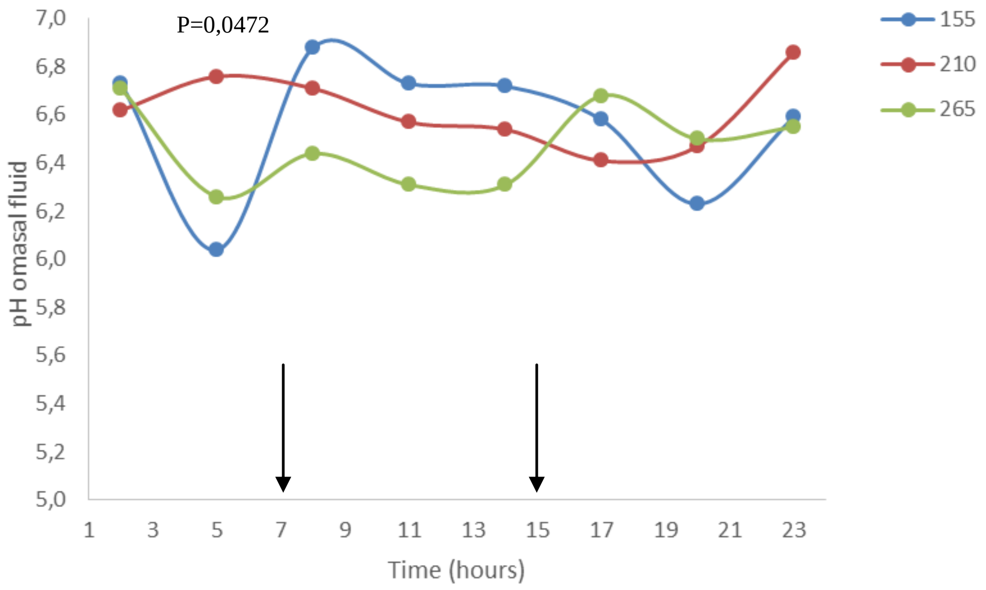

Figure 6. pH of omasal fluid for different gestation times of Holstein heifers.