

VINÍCIUS DE SENA SALES VIANA

**EFFECTS OF DISTILLERS DRIED GRAINS ASSOCIATED WITH HIGH AND
LOW FORAGE FIBER LEVEL ON PERFORMANCE OF DAIRY COWS**

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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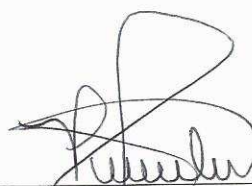
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“Eu sou apenas um rapaz latino-americano
Sem dinheiro no banco
Sem parentes importantes
E vindo do interior
Mas trago de cabeça uma canção do rádio
Em que um antigo compositor baiano me dizia
Tudo é divino, tudo é maravilhoso”

(Antônio Carlos Belchior)

BIOGRAPHY

VINÍCIUS DE SENA SALES VIANA, son of Vasco Sales Viana and Berenice Rodrigues de Sena, was born in Nova Russas/CE-Brazil on September 16, 1993.

Viana, bachelor's start degree in Animal Science at the Universidade Federal do Ceará in 2013 and obtained Bachelor of Science in Animal Science in December of 2017.

On November of 2020, Viana defended his master's dissertation to obtain the Magister Scientiae degree in Animal Science.

ABSTRACT

VIANA, Vinicius de Sena Sales, M.Sc., Universidade Federal de Viçosa, November, 2020. **Effects of Distillers Dried Grains Associated with high and low forage fiber level on Performance of Dairy Cows.** Advisor: Polyana Pizzi Rotta. Co-advisors: Marcos Inácio Marcondes, Edenio Detemann and Sebastião Campos Valadares Filho.

The objective of this study was to evaluate the effects of increasing levels of distillers dried grains (DDG) in high and low forage neutral detergent fiber (NDFf) levels on intake, digestibility, feed sorting behavior, milk yield and composition, hormonal profile, and economic analysis of the diets. Twelve Holstein cows were assigned to a replicated 6×6 Latin squares with a 3×2 factorial arrangement of treatments. Diets were formulated with 0, 20, and 40% DDG on dry matter (DM) basis with low forage (21% NDFf) or high forage (31% NDFf). Cows fed diet with lower inclusion level of NDFf had the greatest DM intake. Dry matter intake and other nutrients intake were lower at inclusion of 40% DDG than 0 or 20% DDG. Dry matter, organic matter, and crude protein digestibility was inversely proportional to increasing DDG levels. In addition, there was a decrease in crude protein ruminal digestibility when increasing DDG level. The cows submitted to the treatments with 40% of inclusion of DDG refused the concentrated ration of the diet. Distillers dried grains did not affect ruminal pH. The inclusion of up to 20% DDG does not affect the intake and digestibility of dairy cows. About milk yield, we observed similar results for cows fed diets with 0 and 20% of DDG. Milk protein decreased with increasing inclusion of DDG in the diet. We observed no effect of diets for milk fat concentration. We also did not observe difference for microbial protein synthesis among treatments. We found a reduction in plasma glucose with an increase of DDG level. Total plasma cholesterol concentration increased with the greater level of DDG in diet. However, plasma urea concentration was reduced with the increase of DDG in diet. The nitrogen balance presented a negative value for all treatments, except for the animals fed with 0% DDG and the lowest NDFf level. According to income over feed cost analysis, we observed an economic advantage when feeding 20% DDG diets compared with 0 and 40% DDG diets. The inclusion up to 20% DDG does not affect the productive performance of dairy cows and may be an alternative of feeding dairy cows.

Keywords: Alternative feeds. Coproducts etanol. Dairy yield.

RESUMO

VIANA, Vinicius de Sena Sales, M.Sc., Universidade Federal de Viçosa, novembro de 2020. **Efeitos de grãos secos de destilaria associado a baixa ou alta fibra proveniente da forragem no desempenho de vacas leiteiras.** Orientador: Polyana Pizzi Rotta. Coorientadores: Marcos Inácio Marcondes, Edenio Detemann e Sebastião Campos Valadares Filho.

O objetivo deste estudo foi avaliar os efeitos de níveis crescentes de grãos secos de destilaria (DDG) em diferentes níveis de fibra em detergente neutro de forragem (FDNf) sobre o consumo, digestibilidade, comportamento de seleção de alimentos, desempenho e composição do leite, perfil hormonal e análise econômica das dietas. Doze vacas Holandesas foram distribuídas em quadrados latinos 6×6 replicados com arranjo fatorial 3×2 de tratamentos. As dietas foram formuladas com 0, 20 e 40% de DDG na matéria seca (MS) com baixa FDN da forragem (21% FDNf) ou alta (31% FDNf). Vacas alimentadas com dieta com menor nível de inclusão de FDNf tiveram maior ingestão de MS. O consumo de matéria seca e outros nutrientes foram menores com a inclusão de 40% DDG do que 0 ou 20% DDG. A digestibilidade da matéria seca, matéria orgânica e proteína bruta reduziu com a inclusão de DDG. Além disso, houve redução da digestibilidade ruminal da proteína bruta com o aumento do nível de DDG. A inclusão de até 20% de DDG não afeta o consumo e a digestibilidade das vacas leiteiras. Quanto à produção de leite, observamos resultados semelhantes para vacas alimentadas com dietas com 0 e 20% de DDG. A proteína do leite diminuiu com o aumento da inclusão de DDG na dieta. Não observamos efeito das dietas para a concentração de gordura do leite. Também não observamos diferença para síntese de proteína microbiana entre os tratamentos. Encontramos uma redução na glicose plasmática com um aumento do nível de DDG. A concentração de colesterol plasmático total aumentou com o maior nível de DDG na dieta. No entanto, a concentração de uréia plasmática foi reduzida com o aumento de DDG na dieta. O balanço de nitrogênio apresentou valor negativo para todos os tratamentos, exceto para os animais alimentados com 0% DDG e menor nível de FDNf. De acordo com a análise sobre o custo da alimentação, observamos uma vantagem econômica ao alimentar dietas com 20% de DDG em comparação com dietas com 0 e 40% de DDG.

Palavras-chave: Alimentos alternativos. Coprodutos do etanol. Produção de leite.

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GENERAL INTRODUCTION

Sugarcane is the main source of feedstock for ethanol production in Brazil, however, others alternatives to have been explored in the industry. Total ethanol production from corn has been growing, and should reach 1.4 billion liters in the 2019/20 harvest, an increase of more 70% in relation to the previous year (Barros, 2019).

The demand for the generation of energy from renewable sources has increased the interest of countries to develop alternative technologies to fossil fuels, aiming at reducing the emission of pollutants and the intention to generate energy in a more sustainable way (EIA, 2012; Milanez et al., 2015; Silva, Netto and Scussel, 2016).

Among the alternatives we have the production of fuels from vegetable biomass: ethanol. The increase in ethanol production from corn results in a greater availability of dry distillery grains (DDG), which has a high concentration of protein and low cost in relation to other protein sources in concentrated feed. The use of agro-industrial by-products in the feeding of ruminants, in addition to promoting a reduction in the cost of feeding, reuses organic matter of plant origin from industry, reducing waste accumulation and environmental contamination, collaborating with the preservation of natural resources and promoting sustainability to animal production (Aldai et al., 2010; Rodrigues and Rondina, 2013).

Therefore, this experiment to goal to evaluate the effects on intake, digestibility, sorting behavior, milk yield and milk composition, hormonal profile in plasma, and economic analysis of the diets, with the inclusion of DDG in the feeding of lactating cows at different levels (0, 20 and 40%, in dry matter of diet) and with high and low concentration of NDFf (< 21% and > 31%).

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CHAPTER 1

INTERPRETATIVE SUMMARY: Effects of Distiller-Dried Grains on Productive Responses of Dairy Cows: **I. Intake, Sorting, and Digestibility**

Viana et al. The objective of the current study was to evaluate the effects of levels of dried distilled grains on intake, digestibility and sorting profiles in dairy cows. This study demonstrated a reduction in the DMI only with diets containing 40% DDG. In addition, there was a decrease in CP digestibility for diets with 40% DDG. The cows submitted to the treatments with 40% DDG refused the concentrated ration of the diet. However, DDG does not affect the ruminal pH, generating great stability in the ruminal environment of the cows even after feeding periods.

Running head: DISTILLER-DRIED GRAINS**Effects of Distiller-Dried Grains Associated with High and Low Forage Fiber Levels on the Performance of Dairy Cows. Cows: I. Intake, Digestibility, and Sorting****V. S. S. Viana, M. I. Marcondes, S. C. Valadares Filho, E. Detmann, J. M. V.****Pereira, V. C. L. Morais, B. C. Silva, D. F. Quirino and P. P. Rotta¹**

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ABSTRACT

The objective of this study was to evaluate the effects of increasing levels of distiller-dried grains (DDGs) in high- and low-forage neutral detergent fiber (NDFf) level diets on intake, digestibility, and sorting in dairy cows. Twelve Holstein cows were assigned to replicated 6×6 Latin squares with a 3×2 factorial arrangement of treatments. Diets were formulated with 0, 20, and 40% DDG on a dry matter (DM) basis with low forage (21% NDFf) or high forage (31% NDFf). Cows fed a diet with a lower inclusion level of NDFf had the greatest DM intake. Dry matter intake and other nutrient intakes were lower with the inclusion of 40% DDG than at 0 or 20% DDG. Dry matter, organic matter, and crude protein digestibility were inversely proportional to increasing DDG levels. In addition, there was a decrease in crude protein ruminal digestibility with increasing DDG levels. The cows submitted to the treatments with 40% DDG refused the concentrated ration of the diet. Distiller-dried grains did not affect the ruminal pH. The inclusion of up to 20% DDG does not affect the intake and digestibility of dairy cows and may be an alternative for feeding dairy cows.

Keywords: digestibility, intake, forage fiber, industrial coproducts, sorting.

INTRODUCTION

Brazil's 2019 ethanol production was estimated to reach 34.5 billion liters, with an increase of 4% compared to 2018 (Barros, 2019). Sugarcane is the main source used for ethanol production in Brazil; however, other alternatives have been explored in the industry. The total ethanol production from corn has been growing and should reach 1.4 billion liters in the 2019/2020 harvest, with an increase of more than 70% per year.

The ethanol yield from sugarcane has greater productivity per unit land area than that from corn; however, the sugarcane harvest is seasonal, and sugarcane is not available throughout the year since it is a perennial crop but is harvested annually. Thus, the use of corn in the production of ethanol emerges as an advantageous strategy for ethanol plants in the event of a shortage of sugarcane at certain times of the year. Therefore, distiller-dried grains (**DDGs**) are a novelty in Brazil, with the potential to increase the availability of coproducts. Thus, studies using DDG to feed lactating cows are important and necessary to extend the possibility of its use.

One of the biggest challenges to a greater acceptability of DDGs in the livestock industry nationally and internationally is the wide variability in the composition and digestibility of nutrients (Kalscheur et al., 2012). This variability is the result of differences in the corn used to produce ethanol and differences in the production process, types of yeast, fermentation, distillation efficiency, drying processes, and amount of soluble substances mixed with the coproducts (Liu, 2011; Zanton et al., 2013).

Another concern related to the inclusion of greater amounts of coproducts from the ethanol industry is the possibility of causing a decrease in the concentration of milk fat (Ranathunga et al., 2018). Recent studies have sought to investigate the effect of including coproducts from the low-fat ethanol industry or adding forage NDF to reduce the risk of

depression of milk fat (Ramirez-Ramirez et al., 2016; Ranathunga et al., 2018). Corn distillery grains contain large amounts of neutral detergent fiber (NDF) and have low lignin contents; however, due to their small particle size, they do not function as an effective fiber, so they would have no effects on rumen buffering.

Therefore, the objective of the current study was to evaluate the effects of DDG inclusion (0, 20 and 40% of dietary DM) with low- or high-forage neutral detergent fiber (NDFf; 21 and 31%) level diets on intake, digestibility, and sorting by dairy cows. The hypothesis is that feeding a high DDG (40% inclusion) level with a low NDFf would decrease intake and digestibility and alter the sorting profile.

MATERIALS AND METHODS

Location of the Study

The study was conducted at the Dairy Unit of the Teaching and Research Farm of the Department of Animal Science, Universidade Federal de Vicosa (UFV), Brazil. Universidade Federal de Vicosa is in the municipality of Viçosa in the state of Minas Gerais, located at latitude 20° 45 '14 "S and longitude 42° 52' 55" W, with a 663 m altitude. The region's climate is characterized as humid subtropical, according to the KÖPPEN-GEIGER (Cwa) climate classification. The average annual temperature in the municipality is 20.6°C, with an average annual rainfall of 1229 mm.

Ethical Considerations

All procedures involving the use of animals were previously submitted to the Ethics Committee for the Use of Research Animals of Universidade Federal de Viçosa for approval under protocol number 09/2019.

Animals, Diets, and Experimental Design

Twelve primiparous Holstein dairy cows (27 ± 2.8 months) with an initial DIM of 99 ± 24.5 and an initial body weight of 502 ± 27.9 kg were used in an incomplete replicated 6×6 Latin square design arranged in a 3×2 factorial, with six treatments including three DDG and two NDFf levels. Six animals were cannulated in the rumen to assess ruminal and intestinal digestibility. The experiment had five experimental periods of 25 d each, with the first 14 d for cow adaptation and 11 d for data and sample collection. The average milk production was 30.5 ± 1.36 kg/d. Throughout the study, the cows were housed in individual tie-stalls and had free access to water. Cows were fed experimental TMR three times a day at 0700, 1500, and 2000 h at 110% of the actual feed intake of the previous day. The weight of feed offered and refused was recorded daily for all cows. The cows were milked three times a day at 0630, 1430, and 1930 h.

The experimental diets were formulated to contain 0, 20, or 40% DDG with low or high NDFf (21 or 31%; Table 1). The rations were formulated using Dairy NRC (2001) to meet or exceed all nutrient requirements for a 550 kg cow producing 30 kg/d of milk with 3.5% milk fat and 3.0% milk protein.

The ethanol byproduct used in this experiment was supplied by a commercial company in Brazil, with a crude protein content of 30%, an ethereal extract of 5%, ash of approximately 2%, neutral detergent fiber (NDF) of 46%, fiber in acid detergent (FDA)

of 21%, fiber in indigestible neutral detergent (iNDF) of 7%, and 5.5% starch. The dry matter (DM) content was approximately 91%.

Sample Collection

Forage, concentrate, and TMR samples were collected on the 17 to 20th day of each experimental period and stored at -20°C until analysis. Feed samples were dried in a forced-air oven at 55°C for 72 h. During the experiment, three batches of concentrate mixes were prepared for each treatment at the university feed mill. Individual ingredients, namely, DDG, ground corn, soybean meal, bypass soybean meal, cottonseed and mineral mixture, from the same batches were sampled each time the concentrates were mixed in the feed mill. All feed samples were dried at 55°C in the same forced-air oven for 72 h and then ground through a 2 mm screen using a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA). Samples were further ground through a 1 mm screen.

The animals were subjected to feces and urine collection for four consecutive days on the 18th to 21st day of each experimental period, 12 h apart, totaling 8 samples for each animal per experimental period (Figure 1). The feces were collected directly from the animal's rectum and placed in an aluminum tray for drying in an oven with forced ventilation at 55°C for 72 h. After the drying period, the samples from the four days of collection were ground in a knife mill fitted with 1 and 2 mm screens, and composite samples were made proportional to the total dry weight of the feces from each collection day, which were placed in plastic pots.

Five days before omasal digesta sampling, 7.0 g/d Co-EDTA was diluted in 4 L distilled water and continuously infused into the omasum by a ruminal fistula using a peristaltic pump (BP-model. 600.4; Milan Scientific Equipment, Colombo, Parana,

Brazil). Six collections of omasal digesta were performed at 12-h intervals, totaling three collection days per experimental period.

The ruminal and intestinal digestibility was estimated by the digestion technique developed by Faichney (1975) using the double marker system. The use of the dual marker system is well established, and its use is extensively documented in several studies (Faichney, 1975; France and Siddons, 1986; Rotta et al., 2014). Measurements of the digestion flow using the dual marker system are based on the use of cobalt as the liquid phase marker and NDFi as the particle phase marker. The reconstitution factor was calculated based on the concentrations of the markers at the different stages of digestion (France and Siddons, 1986).

Chemical Analysis of Samples

All feed samples were evaluated for DM according to the INCT method - CA G-003/1, mineral matter (**MM**) according to the INCT method – CA M-001/1, CP according to the INCT method - CA N-001/1, and NDF according to the INCT method - CA F-001/1. Ether extract (**EE**) was analyzed using the Randall method according to the INCT method - CA G-005/1. The quantification of NFC was performed according to Detmann et al. (2012) using the following equation:

$$\text{NFC} = 100 - [(\% \text{CP} - \% \text{Urea CP} + \% \text{Urea}) + \% \text{NDF} + \% \text{EE} + \% \text{MM}].$$

Sorting

The evaluation of feed selection by cows was carried out by collecting the orts of each animal, which were homogenized, and a sample was subsequently taken for chemical

analysis. The other part of the sample was weighed and subjected to the Pennsylvania State Particle Separator (**PSPS**).

The PSPS was developed at the University of Pennsylvania (USA), consisting of three sieves (19, 8 and 4 mm) and a smooth bottom to produce long (**P1**; > 19 mm), medium (**P2**; < 19 > 8 mm), short (**P3**; < 8 > 4 mm) and very short (**P4**; < 4 mm) particles. On a smooth surface, the PSPS particle separator set was assembled in the following order: smooth bottom, a 4 mm sieve, an 8 mm sieve and, finally, a 19 mm sieve. Next, samples from the respective experimental diets and orts of each animal in each period were placed on top of the 19 mm sieve, and then separation movements were started, totaling 40 back-and-forth movements. The particle sizes of the experimental diets offered and orts were estimated consecutively over the three days of observation of the animals in each evaluation period to assess the selection of the diet components. The particle size estimates were expressed as the percentages of particles retained in the different sieves.

The data on the contents of NDF, CP, and NFC in feedstuffs and refusals were calculated as the actual intake of each fraction in relation to the predicted intake. Values <100% indicated selective refusals (sorting against), values >100% indicated preferential intake (sorting for), and values = 100% indicated no sorting (Leonardi and Armentano, 2003; Cunha et al., 2019).

Statistical Analysis

All data were analyzed by Statistical Analysis System® software. The design of this experiment was a replicate 6 × 6 incomplete Latin square with a 3 × 2 arrangement of treatments. The data were analyzed with the experimental model:

$$Y_{ijkl} = \mu + Q_i + P_j + A(Q)_k + F_l + DDG_m + F_l \times DDG_m + e_{ijkl}$$

where Y_{ijkl} = observed value; μ = overall mean; Q_i = Latin square effect i , $i = 1$ to 2 ; P_j = effect of period j , $j = 1$ to 5 ; $A(Q)_k$ = effect of animal k within each Latin square, $k = 1$ to 12 ; F_l = effect of NDFf l , $l = 1$ to 2 ; DDG_m = effect of DDG inclusion levels m , $m = 1$ to 3 ; $F_l \times DDG_m$ is the interaction between the NDFf and the DDG; and e_{ijkl} is the random error associated with each observation. The NDFf, DDG and interaction between them were analyzed as fixed effects, and the Latin square, period and animal were considered random effects.

The ruminal and omasal pH were analyzed as repeated measures, considering the effect of the time of sample collection, as follows:

$$Y_{ijkl} = \mu + Q_i + P_j + A(Q)_k + F_l + DDG_m + T + F_l \times DDG_m + T \times F_l + T \times DDG_m + T \times F_l \times DDG_m + \varepsilon_{ijkl},$$

where Y_{ijkl} = observed value; μ = overall mean; Q_i = Latin square effect i , $i = 1$ to 2 ; P_j = effect of period j , $j = 1$ to 5 ; $A(Q)_k$ = effect of animal k within each Latin square, $k = 1$ to 12 ; F_l = effect of NDFf l , $l = 1$ to 2 ; DDG_m = effect of DDG inclusion levels m , $m = 1$ to 3 ; T = effect of time; $F_l \times DDG_m$ is the interaction between the NDFf and the DDG; $T \times F_l$ is interaction between the time and NDFf level; $T \times DDG_m$ is interaction between the time and DDG levels; $T \times F_l \times DDG_m$ is interaction between the time, NDFf levels and DDG levels; and e_{ijkl} is the random error associated with each observation.

For all analyses, multiple comparisons between treatments were performed using the "t" test, and differences were declared when $P < 0.05$.

RESULTS

Nutrient Intake

The level of NDFf influenced the DMI and all the nutrients evaluated. Cows submitted to treatments with low NDFf had a greater DMI ($P = 0.04$), OM ($P = 0.03$), EE ($P < 0.01$), and TDN ($P < 0.01$), whereas for NDF intake, the greatest value was observed for cows submitted to treatments with lower concentrations of NDFf ($P < 0.01$). We also observed an effect of DDG on the DMI ($P < 0.01$), OM ($P < 0.01$), NDF ($P < 0.01$), EE ($P < 0.01$), and TDN ($P < 0.01$). For the DM, OM, and TDN, we observed a decreasing quadratic effect ($P < 0.01$), and for NDF, we observed an increasing quadratic effect ($P = 0.01$).

There was an interaction ($P < 0.05$) between NDFf and DDG for CP intake, with a decreasing linear effect ($P < 0.01$) for treatments with low NDFf concentrations and a decreasing intake according to increased DDG levels (Figure 2). For treatments with high concentrations of NDFf, no difference ($P = 0.06$) in CP intake with the inclusion of DDGs was observed. There was an interaction between NDFf and DDG for NFC intake, with a decreasing quadratic effect ($P < 0.05$) for treatments with high and low concentrations of NDFf with decreasing DDG (Figure 3).

Apparent Total-Tract, Ruminal, and Intestinal Digestibility

We observed an effect of NDFf on DM, OM, CP, EE, and NFC digestibilities, with greater values for cows submitted to treatments with lower concentrations of NDFf (Table 3). Distiller-dried grain levels affected DM ($P < 0.01$), OM ($P < 0.01$), and CP ($P < 0.01$) digestibility, with a linear decreasing effect with the inclusion of DDG in the diets.

For treatments with low NDFf, we observed an increasing linear effect for NDF digestibility as the level of DDG increased ($P < 0.01$). For treatments with high NDFf, we observed a decreasing linear effect ($P = 0.01$), with a reduction in NDF digestibility according to increasing DDG in the diet (Figure 4).

The NDFf level affected ($P < 0.01$) the ruminal digestibility of DM, OM and CP, with reduced ruminal digestibility in treatments with greater inclusion of NDFf. We also observed a quadratic effect ($P = 0.02$) of DDG levels on NDF ruminal digestibility, with lower ruminal digestibility for treatments with DDG inclusion. A decreasing linear effect ($P = 0.03$) was observed for the intestinal digestibility of CP, with a lower digestibility with the inclusion of DDG in diets.

Ruminal and Omasal pH

We observed effects of time and DDG levels for the pH value ($P < 0.01$; Figure 5). The lowest pH value occurred at 0100 h (5.96), and the greatest pH value occurred at 0900 h (6.28). Regarding DDG inclusion levels, a quadratic effect ($P < 0.01$) of DDG was observed, in which the pH values were greater for 40% DDG in the DM of the diet (Figure 5).

Sorting

We observed an interaction ($P > 0.05$) between NDFf and DDGs only for the P1 percentages of the particle separator sieves (Table 4). There was no effect ($P > 0.05$) of NDFf on the particle separator sieves. However, for the DDG level, an effect was observed for only the P4 sieve, with an increasing linear effect for P4. Therefore, there was an increase in the concentration of feed in the orts (very short particles; < 4 mm) of cows subjected to the inclusion of DDG in the diet.

Based on sorting behaviors, an effect of NDFf levels on NDF composition was observed, with a more pronounced refusal for the treatments with greater levels of NDFf

(sorting for NDFf = 0.96 vs 0.98; $P < 0.05$). There was a decreasing linear effect ($P < 0.001$) for CP with refusal in treatments with a greater level of DDG (Table 4). There was also an increasing linear effect ($P = 0.02$) for NFC preference in treatments with greater inclusion of DDGs (sorting for NFC = 1.08).

DISCUSSION

The reduction in the intake of OM, CP, EE, NFC, and TDN for cows fed with the greater NDFf level occurred due to the lower DMI. Diets with high levels of NDFf had a greater concentration of NDF in the diet with an average increase of 25% compared to diets with a low level of NDFf.

Ferraretto et al. (2013) conducted a meta-analysis study showed that DMI of dairy cows decreased with increasing NDFf concentration in the diet, due to the filling effect of forages. In the present study, the decrease in DMI of cows fed the high NDFf diet likely resulted from longer retention time of forage particles, restricting the flow of digesta through the gastrointestinal tract (Allen, 1996; Oba and Allen, 2003; Li et al., 2020). Other authors also observed increasing concentration of NDFf in the diet decreases DMI due to the filling effect of the forage on the rumen (Fredin et al., 2015). The greater intake of NDF in diets with a greater level of NDFf occurs due to the increased concentration of NDF in the diets with the increased inclusion levels of NDFf.

Regarding the level of DDG, the reduction in DMI occurred only in cows fed diets containing 40% DDG. The decrease in DMI observed may have been limited by the lower acceptability of DDGs to cows. The low acceptability of DDGs to cows may be associated with severe heat damage during industrial processing (Almeida et al., 2013; Böttger and Südekum, 2018).

The reduction in CP intake for treatments with the lowest level of NDFf in the diet and 40% inclusion of DDG may have occurred due to the reduction in DMI; moreover, the cows refused the concentrated feed, as confirmed by the greater concentration of very small particles in theorts. The reduction in NCF intake in diets with 40% DDG may have occurred due to the DMI reduction, as well.

A decrease in DMI was not observed in previous studies in which dairy cattle were fed diets with up to 30% DDG (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016). Dry matter intake was reduced by more than 11% when 40% DDG was provided. DMI is especially important for milk production, as cows with a greater DMI produce more milk (NRC, 2001; Reshalaitihan et al., 2020).

The digestibility of DM and OM decreased with increasing NDFf, mainly due to the lower digestibility of CP, EE, and NCF as the dietary levels of NDFf increased. The greater forage NDF and lower NFC content of diets with greater NDFf levels may have contributed to this result. Alves et al. (2016) suggested a linear reduction in DM digestibility with increased rates of forage inclusion. In addition, the main forage source in this study was corn silage and tifton hay, which have a lower digestibility than that of alfalfa- and barley-based diets, for example. Compared with corn-based diets, barley-based diets resulted in improved fiber digestion. Li et al. (2020) reported that barley-based diets resulted in improved fiber digestion compared with corn-based diets.

Regarding the level of inclusion of DDG, the decrease of more than 10% in CP digestibility with the inclusion of DDG shows a lower quality of the protein present in the DDG used in the experimental diets. The reduction in the digestibility of CP with increasing DDG in diets may indicate that the DDG was dried under more extreme conditions, which may have increased the amount of protein potentially unavailable to

the animal (Kleinschmit et al., 2006). The reduction in the intestinal digestibility of CP with the increase in DDG levels in cow diets reinforces the low quality of CP.

There is a strong possibility that excessive heat treatment during the drying process of DDGs may have decreased the availability of the protein (NRC, 2001; Schingoethe et al., 2009; Acharya et al., 2015). However, Anderson et al. (2015) demonstrated that diets with a high content of DDG with solubles improved the digestibility of CP and NDF when compared with a mixture of control concentrate based on corn and soybean meal. However, the great variability in the composition of the DDG may lead to different results.

The interaction between NDFf and DDGs for NDF digestibility showed an increase in NDF digestibility with increasing inclusion levels of DDGs and a lower inclusion level of NDFf. This suggests that the cows consumed more digestible NDF. In treatments with high NDFf, the digestibility of NDF was reduced, probably due to the amount of indigestible fiber from the forage, which may have limited the intake of these cows.

The data obtained from the sorting show that cows subjected to the treatments with 40% inclusion of DDG refused the concentrate ration of the diet in preference of the NFC. In addition, during the execution of the experiment and collection of samples, we observed that visually, in the treatments with 40% DDG, there was an ort of concentrated feed. According to Oliveira et al. (2013), increased NFC intake may hinder the action of cellulolytic bacteria in the digestion of NDF, which leads to a reduction in the respective digestibility. The ruminal digestibility of DM, OM, and CP decreased linearly due to the greater concentration of NDFf, which presents a greater number of components with low digestibility or indigestibility.

Despite the pH value varying with the measurement time, a relatively small difference in the value of the ruminal pH (5.96 at 0100 h vs 6.28 at 0900 h) was observed, showing

great stability in the ruminal environment of the cows even after feeding periods. pH thresholds are currently the most appropriate measure for SARA, which is characterized by intermittent periods of reduction in ruminal pH, with pH values ranging from 5.5 to 6.0 (Calsamiglia et al., 2012; Oetzel, 2017). The experimental diets were well buffered with sodium bicarbonate and magnesium oxide. Moreover, cows were fed three times per day, which may have generated less variability in the rumen and omasal pH (Johnston and DeVries, 2018).

Regarding DDG levels, the greater ruminal pH value of cows fed diets with 40% DDG may be explained by the lower DMI and the partial or total replacement of grains in diets. Replacing starch with digestible fiber from nonfodder sources and diets with lower contents of readily fermentable carbohydrates can result in a greater rumen pH (Mao et al., 2013). As dietary starch decreases, less NDFf is required to achieve an adequate pH (White, et al., 2017).

The data obtained by the PSPS showed a linear increase in the percentage of very short particles (< 4 mm) in orts of cows subjected to treatments containing DDG, showing a refusal of the concentrated feed that contained DDG. The data obtained from the sorting may suggest a possible refusal of DDG by the animals, since the cows preferred CP in the treatments with 0 or 20% DDG.

It is possible that there might have been low (or excessive) heat treatment of the DDGs during industrial processing, thus causing protein denaturation in the coproduct and thereby reducing the nutrient digestibility of the batch of DDGs used in the experiment. Some authors have already reported damage from excessive heat in coproducts of the ethanol industry, which impaired the protein quality and amino acid content, mainly affecting lysine (Schingoethe et al., 2009; Paz and Kononoff, 2014; Acharya et al., 2015).

CONCLUSIONS

This study demonstrates a reduction in DMI only with diets containing 40% DDGs, probably limited by the lower acceptability of DDGs. In addition, there was a decrease in CP digestibility for diets with 40% DDG. The cows refused the concentrated ration of the diet when submitted to the treatments with 40% DDG. However, DDG does not affect the ruminal pH, generating great stability in the ruminal environment of the cows even after feeding periods.

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Table 1. Ingredients used in the formulation of experimental diets and composition of diets.

Item	Low NDFf ¹ (21%)			High NDFf (31%)		
	0DDG	20DDG	40DDG	0DDG	20DDG	40DDG
Ingredient (% of DM ³)						
Corn silage	40.4	41.0	41.0	41.2	40.6	40.7
Tifton hay	0.0	0.0	0.0	13.6	14.3	14.3
DDG ²	0.0	21.9	41.7	0	21.0	41.0
Dried ground corn	22.9	17.8	12.0	11.3	8.40	0.0
Soybean meal	17.0	7.80	0.0	14.6	4.70	0.0
Soybean meal bypass	7.08	4.30	0.00	6.93	4.20	0.0
Cottonseed	7.55	2.20	0.00	7.63	2.00	0.0
Sodium bicarbonate	1.20	1.20	1.20	1.20	1.20	1.20
Magnesium oxide	0.60	0.60	0.60	0.60	0.67	0.60
Dicalcium phosphate	0.70	0.60	0.27	0.55	0.63	0.20
Limestone	1.30	1.40	1.62	1.20	1.20	1.42
Urea	0.00	0.19	0.37	0	0.26	0.00
Vitamin E	0.05	0.05	0.05	0.05	0.05	0.05
Premix Mineral	0.83	0.70	0.98	0.83	0.87	0.56
Nutrient (% of DM unless noted)						
DM ³ (% of diet)	48.0	47.6	47.7	47.5	47.9	49.3
Crude protein	17.9	17.9	17.7	17.0	16.8	16.7
RDP ⁴	9.60	9.60	9.70	9.20	9.10	8.90
RUP ⁵	8.30	8.30	8.00	7.80	7.80	7.80
NDF ⁶	27.4	34.5	40.7	36.3	43.0	49.6
NDFf	21.2	20.5	20.1	31.8	31.0	30.7
NFC ⁷	73.4	71.1	68.7	70.9	68.9	67.3
Starch	31.3	27.7	23.4	22.8	20.2	14.5
EE ⁸	4.1	3.8	3.9	3.9	3.5	3.6

¹NDFf= fiber insoluble in neutral detergent from the forage;

²DDG = distillers dried grains;

³DM = dry matter;

⁴RDP = rumen degradable protein;

⁵RUP = rumen undegradable protein;

⁶NDF = fiber insoluble in neutral detergent;

⁷NFC = non-fibrous carbohydrates

⁸EE = Ethereal extract.

Table 2. Intake of dry matter and nutrients of cows fed different levels of distillers dried grains and forage NDF.

Item (basis DM ⁴)	Low NDF ¹			High NDF ¹			SEM ³	P-value			
	0DDG ²	20DDG ²	40DDG ²	0DDG ²	20DDG ²	40DDG ²		FDN ¹	NDF ¹ × DDG ²	Linear	Quadratic
DM ⁴ , kg/d	21.32 ^a	21.11 ^a	18.02 ^c	19.15 ^{bc}	20.26 ^{ab}	18.05 ^c	1.46	0.04	0.19	<0.01	<0.01
OM ⁵ , kg/d	20.47 ^a	20.36 ^a	17.49 ^c	18.32 ^{bc}	19.50 ^{ab}	17.42 ^c	1.41	0.03	0.20	<0.01	<0.01
CP ⁶ , kg/d	3.91 ^a	3.59 ^b	2.86 ^e	3.08 ^{de}	3.22 ^{cd}	2.90 ^e	0.25	<0.01	<0.01	<0.01	0.01
NDF ⁷ , kg/d	7.67 ^d	8.72 ^{bc}	8.43 ^c	9.07 ^b	10.24 ^a	9.86 ^a	0.61	<0.01	0.98	0.01	<0.01
iNDF ⁸ , kg/d	8.28 ^c	8.45 ^c	7.55 ^d	10.22 ^{ab}	10.77 ^a	9.99 ^b	0.60	<0.01	0.69	0.10	0.02
EE ⁹ , kg/d	0.89 ^a	0.80 ^b	0.71 ^{cd}	0.73 ^c	0.71 ^{cd}	0.66 ^d	0.06	<0.01	0.07	<0.01	0.54
NFC ¹⁰ , kg/d	8.90 ^a	8.19 ^b	6.40 ^c	6.37 ^c	6.50 ^c	4.85 ^d	0.58	<0.01	0.04	<0.01	<0.01
TDN ¹¹ , kg/d	15.87 ^a	15.22 ^a	12.70 ^b	13.23 ^b	13.25 ^b	11.32 ^c	1.25	<0.01	0.34	<0.01	<0.01

¹ NDF_f = fiber insoluble in neutral detergent from the forage; low = 21%; high = 31%;

² DDG = distillers dried grains;

³ SEM = standard error of the mean.

⁴ DM = dry matter;

⁵ OM = organic matter;

⁶ CP = crude protein;

⁷ NDF = fiber insoluble in neutral detergent;

⁸ iNDF = Indigestible neutral detergent fibers;

⁹ EE = Ethereal extract;

¹⁰ NFC = non-fibrous carbohydrates;

¹¹ TDN = total digestible nutrients.

Table 3. Apparent total-tract, ruminal and intestinal digestibility of cows fed different levels of distillers dried grains and forage NDF.

Item (%)	Low NDF ¹			High NDF ¹			SEM ³	P-value			
	0DDG ²	20DDG ²	40DDG ²	0DDG ²	20DDG ²	40DDG ²		FDNf ¹	NDFf ¹ × DDG ²	Linear	Quadratic
Total-tract digestibility (%)											
DM ⁴	65.74 ^a	63.36 ^b	60.76 ^c	59.65 ^c	55.75 ^d	53.29 ^e	1.56	<0.01	0.62	<0.01	0.66
OM ⁵	68.47 ^a	65.92 ^b	63.15 ^c	63.11 ^c	58.81 ^d	55.86 ^e	1.50	<0.01	0.45	<0.01	0.69
CP ⁶	70.20 ^a	60.15 ^c	52.57 ^d	63.84 ^b	52.34 ^d	47.42 ^e	2.09	<0.01	0.67	<0.01	0.08
NDF ⁷	44.25 ^c	47.07 ^{bc}	47.69 ^b	49.61 ^a	45.68 ^{bc}	45.30 ^{bc}	1.77	0.57	<0.01	0.70	0.73
EE ⁸	88.41 ^a	88.55 ^a	89.13 ^a	85.89 ^{bc}	84.67 ^c	86.67 ^b	1.35	<0.01	0.73	0.46	0.31
NFC ⁹	89.83 ^a	90.04 ^a	90.78 ^a	84.13 ^c	86.96 ^b	84.68 ^c	1.80	<0.01	0.13	0.34	0.09
Ruminal digestibility (%)											
DM ⁴	46.69 ^a	46.11 ^a	42.92 ^b	37.62 ^c	37.35 ^c	34.32 ^d	2.51	<0.01	0.99	0.09	0.46
OM ⁵	51.17 ^a	46.97 ^b	46.14 ^b	40.92 ^{cd}	41.29 ^{cd}	38.82 ^d	2.70	<0.01	0.53	0.09	0.94
CP ⁶	22.39 ^a	21.62 ^a	8.31 ^{bc}	4.05 ^c	5.68 ^{bc}	7.08 ^{bc}	4.88	<0.01	0.11	0.20	0.42
NDF ⁷	41.83 ^a	39.56 ^b	36.92 ^c	43.09 ^a	33.35 ^d	39.29 ^b	2.13	0.64	0.06	0.04	0.03
Intestinal digestibility (%)											
DM ⁴	17.98 ^{bc}	19.30 ^{bc}	20.03 ^b	24.12 ^a	15.98 ^c	16.31 ^c	3.34	0.87	0.07	0.19	0.32
OM ⁵	16.38 ^{bc}	17.95 ^b	18.12 ^b	23.46 ^a	15.15 ^{bc}	14.84 ^c	3.00	0.83	0.03	0.09	0.36
CP ⁶	48.83 ^b	40.96 ^c	44.31 ^{bc}	59.20 ^a	43.11 ^{bc}	39.43 ^c	5.40	0.53	0.31	0.02	0.19
NDF ⁷	4.42 ^b	5.13 ^b	8.15 ^a	4.72 ^b	8.79 ^a	5.26 ^b	1.69	0.75	0.09	0.12	0.29

¹NDFf = fiber insoluble in neutral detergent from the forage; low = 21%; high = 31%;

²DDG = distillers dried grains;

³SEM = standard error of the mean.

⁴DM = dry matter;

⁵OM = organic matter;

⁶CP = crude protein;

⁷NDF = fiber insoluble in neutral detergent;

⁸EE = Ethereal extract;

⁹NFC = non-fibrous carbohydrates.

Table 4. Sorting behavior of cows fed the experimental diets.

Item	Low NDFf ¹			High NDFf ¹			SEM ³	P-value			
	0DDG ²	20DDG ²	40DDG ²	0DDG ²	20DDG ²	40DDG ²		FDNf ¹	NDFf ¹ × DDG ²	Linear	Quadratic
Particulate separator (%)											
P1 ⁴	6.57 ^a	7.33 ^a	0.48 ^c	2.93 ^b	1.94 ^b	1.46 ^c	1.13	<0.01	<0.01	<0.01	0.03
P2 ⁵	1.25 ^a	1.38 ^a	0.95 ^b	1.27 ^a	1.21 ^{ab}	1.02 ^{ab}	0.21	0.86	0.78	0.12	0.28
P3 ⁶	1.39 ^a	1.31 ^a	1.35 ^a	1.28 ^{ab}	1.38 ^a	1.13 ^b	0.17	0.41	0.51	0.42	0.61
P4 ⁷	0.52 ^c	0.60 ^{bc}	0.97 ^a	0.50 ^c	0.72 ^b	0.92 ^a	0.13	0.81	0.68	<0.01	0.48
Sorting behavior (g/g)											
CP ⁸	1.02 ^a	1.00 ^b	0.98 ^c	1.01 ^{ab}	1.01 ^{ab}	0.99 ^{bc}	0.01	0.43	0.47	<0.01	0.39
NDF ⁹	0.95 ^c	0.97 ^{ab}	0.97 ^{ab}	0.97 ^{ab}	0.98 ^a	0.98 ^a	0.01	0.02	0.68	0.18	0.17
NFC ¹⁰	1.05 ^b	1.05 ^b	1.09 ^a	1.05 ^b	1.06 ^b	1.08 ^a	0.01	0.88	0.74	<0.01	0.29

¹NDFf = fiber insoluble in neutral detergent from the forage; adequate = 21%; high = 31%;

²DDG = distillers dried grains;

³SEM = standard error of the mean.

⁴P1 = long particules percentage (> 19 mm);

⁵P2 = medium particules percentage (< 19 > 8 mm);

⁶P3 = short particules percentage (< 8 > 4 mm);

⁷P4 = very short particules percentage (< 4 mm).

⁸CP = crude proein;

⁹NDF = fiber insoluble in neutral detergent;

¹⁰NFC = non-fibrous carbohydrates;

Figures

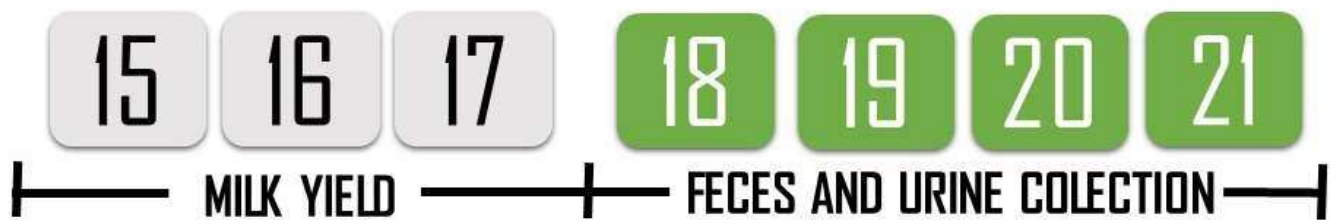
Figure 1. Experimental period scheme.

Figure 2. Interaction between NDFf and DDG for crude protein intake.

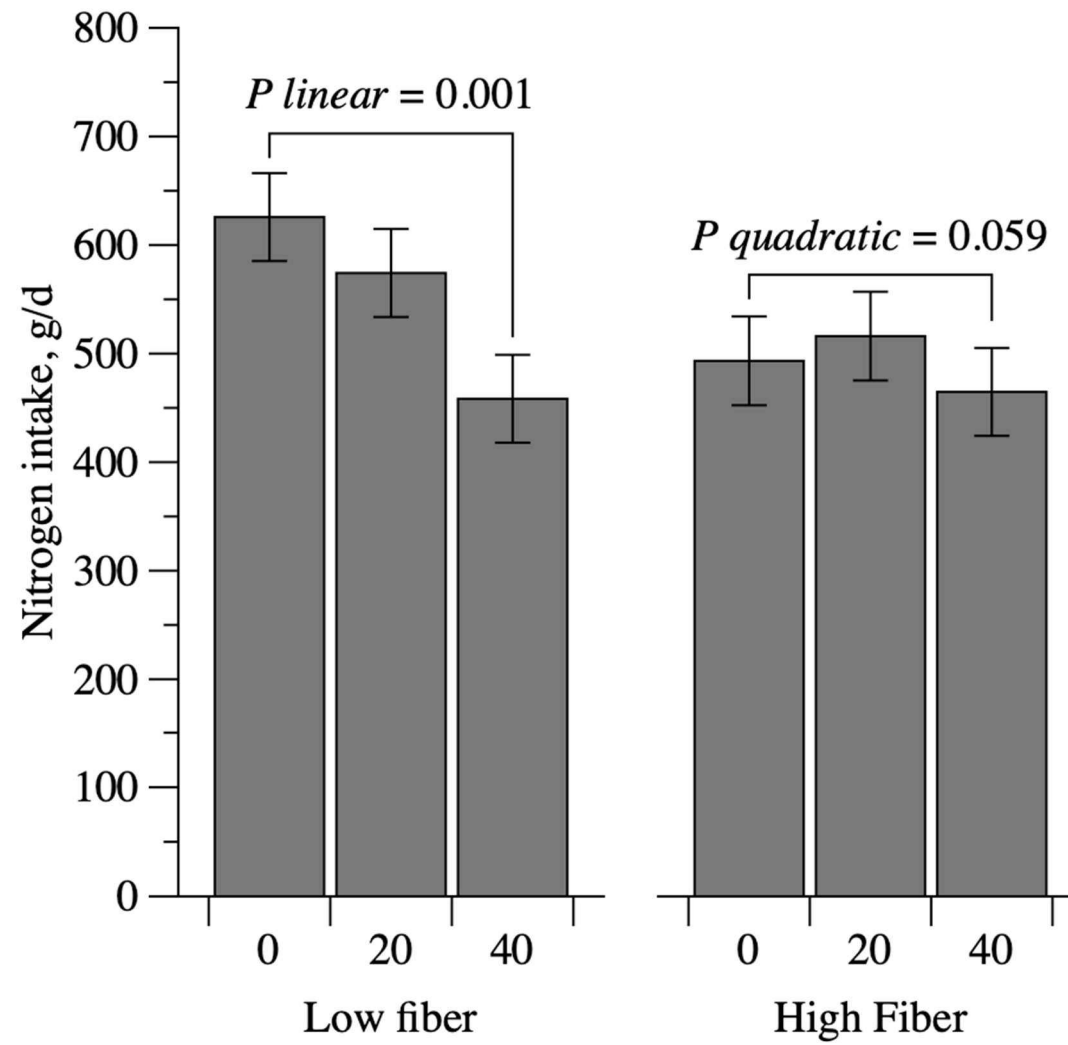
Figure 3. Interaction between NDFf and DDG for non-fibrous carbohydrates intake.

Figure 4. Interaction between NDFf and DDG for digestibility of insoluble fiber in neutral detergent.

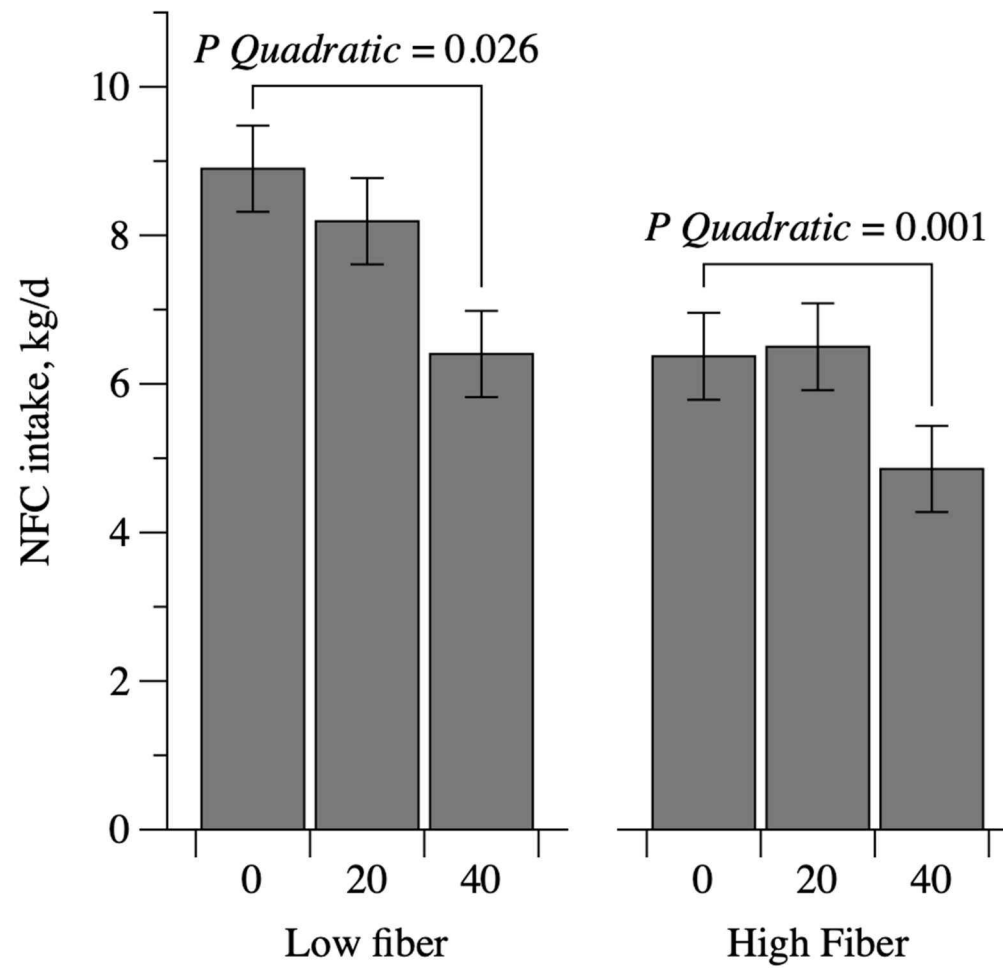
Figure 5. Ruminal and omasal pH value of primiparous cows as a function of measurement time.



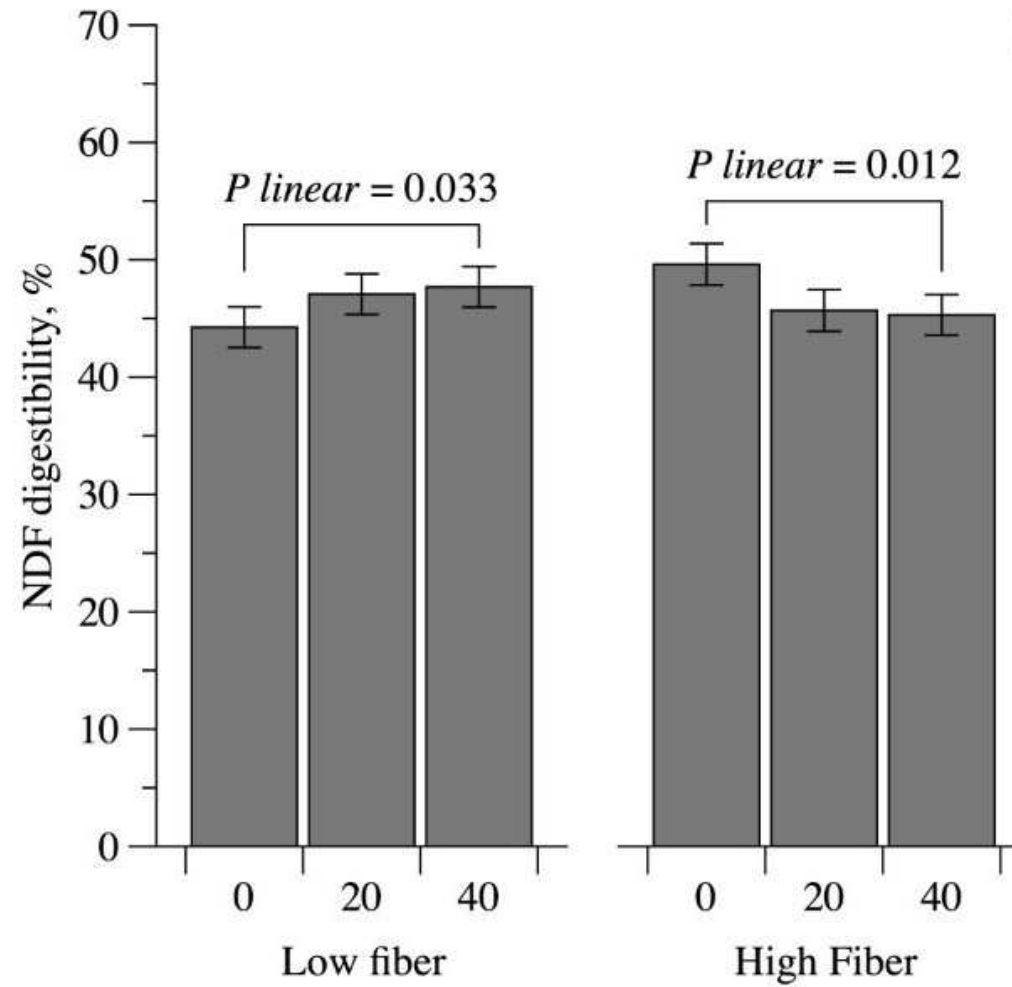
Viana, Figure 1.



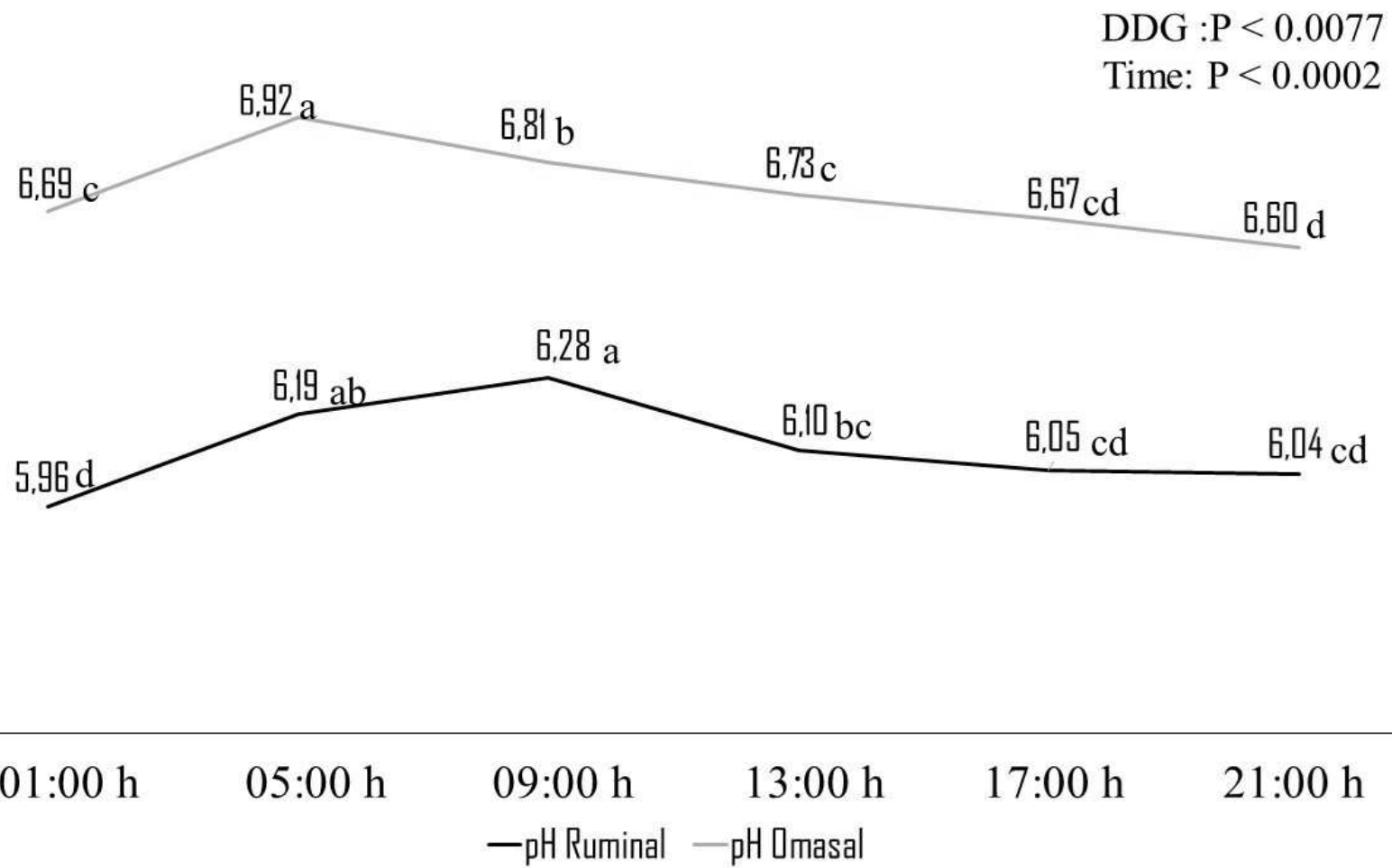
Viana, Figure 2.



Viana, Figure 3.



Viana, Figure 4.



Viana, Figure 5.

CHAPTER 2

INTERPRETATIVE SUMMARY: Effects of Distiller-Dried Grains on the Productive Responses of Dairy Cows: **II. Performance, Hormonal Profile, and Economical Analysis**

Viana et al. The objective of the current study was to evaluate the effects of the levels of distiller-dried grains on the milk production and quality of dairy cows. Feeding a 20% dietary level of dried distilled grains maintained the milk yield and quality compared with no inclusion in the diet. However, the inclusion of 40% DDGs decreased milk production and total solids. The economic analysis of the study showed a decrease in the cost of feed when DDG was included in the diets, showing an economic advantage when feeding 20% DDG.

Running head: DISTILLER-DRIED GRAINS FOR COWS**Effects of Distiller-Dried Grains Associated with High and Low Forage Fiber Levels on the Performance of Dairy Cows: II. Performance, Hormonal Profile, and Economical Analysis****V. S. S. Viana, M. I. Marcondes, S. C. Valadares Filho, E. Detmann, V. C. L.****Morais, J. M. V. Pereira, M. M Ferreira, E. A. C. Lopes and P. P. Rotta¹**

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ABSTRACT

The objective of this study was to evaluate the effects of increasing levels of distiller-dried grains (DDGs) in high- and low-forage neutral detergent fiber (NDFf) level diets on the milk yield and composition, hormonal profiles, and economic analysis of the diets. Twelve Holstein cows were assigned to replicated 6×6 Latin squares with a 3×2 factorial arrangement of treatments. Diets were formulated with 0, 20, and 40% DDG on a DM basis with low forage (21% NDFf) and high forage (31% NDFf). The milk yield was similar to that of cows fed diets with 0 and 20% DDG. The milk protein decreased with increasing inclusion of DDG in the diet. We observed no effect of the diet on the milk fat concentration. We also did not observe differences in microbial protein synthesis among treatments. We found a reduction in plasma glucose with an increase in DDG levels. The total plasma cholesterol concentration increased with increasing DDG levels in the diet. However, the plasma urea concentration was reduced with increasing DDG in the diet. The nitrogen balance presented a negative value for all treatments, except for the animals fed 0% DDG and the lowest NDFf level. According to income-over-feed cost analysis, we observed an economic advantage when feeding 20% DDG diets compared with 0 and 40% DDG diets. The inclusion of up to 20% DDG does not affect the productive performance of dairy cows and may be an alternative for feeding dairy cows.

Keywords: forage fiber, industrial coproducts, milk quality.

INTRODUCTION

The increase in the use of corn for ethanol production generates greater availability of distiller-dried grains (**DDGs**), which are emerging as a good alternative to reduce the costs of feeding cows. Previous studies have reported that DDG may be used at up to 20% DM in the diets of lactating cows, maintaining or increasing milk yields (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016; Ranathunga et al., 2018). Other authors reported greater milk yields with inclusion levels of 30% DDG (Janicek et al., 2008; Abdelqader et al., 2009).

Some problems related to DDG inclusion in dairy cow diets are the possibility of decreasing milk fat concentrations due to its high fat content (Ramirez-Ramirez et al., 2016; Ranathunga et al., 2018). Studies have reported that DDGs may be included in diets with a low concentration of NDF from forage (**NDFf**) without resulting in milk fat depression in dairy cows (Anderson et al., 2006; Kleinschmit et al., 2006; Ranathunga et al., 2018).

Therefore, the primary objective of the current study was to evaluate the effects of DDG inclusion (0, 20, or 40% in DM of diet) with high and low concentrations of NDFf (21 or 31%) on the milk yield and composition, hormonal profiles in plasma, and economic analysis of the diets. We hypothesize that feeding a high-DDG diet with low NDFf would maintain the productive performance and milk composition of lactating dairy cows.

MATERIALS AND METHODS

All procedures involving the use of animals were previously submitted for approval by the Ethics Committee for the Use of Research Animals of Universidade Federal de Viçosa under protocol number 09/2019.

Animals, Diets, and Experimental Design

Twelve primiparous Holstein dairy cows (27 ± 2.8 mo) were used in an incomplete replicate 6×6 Latin square design, with three DDG levels and two NDFf levels. The study had five experimental periods of 21 d each, with the first 14 d for cow adaptation and 7 d for data and sample collection. The cows had an initial DIM of 99 ± 24.5 and initial BW of 502 ± 27.9 kg. The average milk production was 30.5 ± 1.36 kg. The cows were fed experimental TMR three times a day at 110% of the actual feed intake of the previous day. The weights of feed offered and refused was recorded daily. The cows were milk three times per day at 0630, 1430, and 1930 h. The experimental diets were formulated to contain 0, 20, or 40% DDG with low or high NDFf (21 or 31%). For more information about the diets and animal management, please see Viana et al. (2021). The ethanol byproduct used in this experiment was supplied by a commercial company in Brazil, with a crude protein content of 30% and an ethereal extract of 5%.

Sample Collection

Forages, concentrate rations and ort samples were collected for three consecutive days on the 17 to 20th day of each experimental period and stored at -20°C until analysis. Feed samples were dried in a forced-air oven at 55°C for 72 h. During the experiment, three batches of concentrate mixes were prepared for each treatment at the university feed mill.

Individual ingredients including DDG, ground corn, soybean meal, bypass soybean meal and cottonseed from the same batches were sampled each time when the concentrates were mixed at the feed mill. All feed samples were dried at 55°C in a forced-air oven for 72 h and then ground through a 2-mm screen using a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA). Samples were further ground through a 1-mm screen.

The animals were submitted to fecal and urine spot collection for four consecutive days on the 18 to 21st day of each experimental period, 12 h apart, totaling 8 samples for each animal per experimental period (Figure 1). The feces were collected directly from the animal's rectum and placed in an aluminum tray for drying in an oven with forced ventilation at 55°C for 72 h. After the drying period, the samples from the four days of collection were ground in a knife mill at 2 and 1 mm, and composite samples was made proportional to the total dry weight of the feces of each collection day, which were placed in plastic pots. The urine samples were collected with the aid of plastic containers, stimulating the excretion of urine by massaging the vulva of the animals, after which two samples—one of pure urine and one diluted in sulfuric acid at a concentration of 0.036 N—were collected. Then, the urine samples were stored in a freezer at -20 °C.

Chemical Analysis of Samples

All feed samples were evaluated for DM according to the INCT method - CA G-003/1, mineral matter (MM) according to the INCT method – CA M-001/1, CP according to the INCT method - CA N-001/1, and NDF according to the INCT method - CA F-001/1. Ether extracts (EE) were analyzed using the Randall method according to the INCT

method - CA G-005/1. The quantification of NFC was performed according to Detmann et al. (2012) using the following equation:

$$\text{NFC} = 100 - [(\% \text{ CP} - \% \text{ Urea CP} + \% \text{ Urea}) + \% \text{ NDF} + \% \text{ EE} + \% \text{ MM}].$$

Milk Production and Composition

Individual milk yields were recorded at each milking using a mechanical milking electronic flow meter (GEA Westfalia Surge of Brazil, GEA Farm Technologies of Brazil, Indústria e Comércio de Equipamentos Agrícolas e Pecuários Ltda, Jaguariuna, São Paulo, Brazil). The milk yield was also corrected to 3.5% fat according to the formula described by Sklan et al. (1994). Milk samples were collected on three consecutive days from the 15th to 17th day of each period. All collected milk samples were sent to EMBRAPA - Milk Quality Laboratory (Juiz de Fora, Minas Gerais, Brazil) for milk composition analysis. Milk fat, protein, and lactose were analyzed using mid-infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN; AOAC International, 2006), and the concentration of MUN was analyzed using a colorimetric enzymatic method (Bentley Chempeck 150: User's Guide. Chaska, 1998).

The yields of fat-corrected milk and energy-corrected milk were calculated using milk yield and component concentrations from each milking. Fat-corrected milk was calculated as 3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]. Energy-corrected milk was calculated as ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)]. Feed efficiency is one of the most relevant metrics of the efficiency of milk production (Bach et al., 2020). To assess the efficiency of cows in milk production, we used the milk yield over the feed consumed (ECM/DMI).

Economic Analysis of the Diets

In addition to the importance of feed efficiency on profitability, the milk yield is an important factor in farm profitability because as the milk yield increases, the proportion of total farm fixed expenses decreases (Liang and Cabrera, 2015; Bach et al. 2020). For this reason, we used the income over feed cost (**IOFC**) for a more accurate assessment of the economic advantage of using DDGs in feeding cows. The IOFC of the experimental diets was calculated as:

$$\text{IOFC} = (\$/\text{cow per day}) = \text{all-milk price } (\$/\text{kg}) \times \text{daily average milk production} \\ (\text{kg}/\text{cow per day}) - \text{daily feed cost } (\$/\text{cow per day}).$$

Average prices from July 2020 were used for corn silage (\$27.6/t), Tifton hay (\$238.9/t), ground corn (\$0.20/kg), soybean meal (\$0.35/kg), bypass soybean meal (\$0.38/kg), DDG (\$0.16/kg), dicalcium phosphate (\$0.33/kg), limestone (\$0.04/kg), salt (\$0.12/kg), and vitamins (\$0.73/kg). The prices of corn silage and Tifton hay were obtained according to the prices offered in the region. The prices of corn grain, soybean meal and bypass soybean meal were obtained from Cargill Ltd. Inc., Uberlândia, Minas Gerais, Brazil. The prices of all other ingredients were obtained from the Center for Advanced Studies in Applied Economics - CEPEA/Esalq (2020). Milk prices were obtained from CEPEA/Esalq from July 2020, with average prices from July 2020 of \$0.32/kg.

Blood Sampling and Analysis

Blood was sampled from all cows via the tail blood vessels before morning feeding (0630 h) on the last day of each experimental period using coagulation activator tubes and serum separating gel (BD Vacutainer® SST® II Advance®, São Paulo, Brazil) to quantify the urea, triglycerides, total cholesterol, NEFA, IGF-1, and insulin. Tubes with clot activator and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) were used to quantify the plasma glucose concentration. The collected blood samples were immediately placed on ice and transported to the laboratory to be centrifuged.

Statistical Analysis

All data were analyzed by the computer program Statistical Analysis System®. The design of this experiment was a replicate 6×6 incomplete Latin square with a 3×2 arrangement of treatments. The data were analyzed with the model:

$$Y_{ijkl} = \mu + Q_i + P_j + A(Q)_k + F_l + DDG_m + F_l \times DDG_m + e_{ijkl},$$

where Y_{ijkl} = observed value; μ = overall mean; Q_i = Latin square effect i , $i = 1$ to 2 ; P_j = effect of period j , $j = 1$ to 5 ; $A(Q)_k$ = effect of animal k within each Latin square, $k = 1$ to 12 ; F_l = effect of NDFf l , $l = 1$ to 2 ; DDG_m = effect of DDG inclusion levels m , $m = 1$ to 3 ; $F_l \times DDG_m$ is the interaction between the NDFf and the DDG; and e_{ijkl} is the random error associated with each observation. The NDFf, DDG and interaction between them were analyzed as fixed effects, and the Latin square, period and animal were analyzed as random effects. For all analyses, multiple comparisons between treatments were performed using the "t" test, and differences were declared when $P < 0.05$.

RESULTS

Milk Yield and Composition

No interaction ($P > 0.05$) of NDFf \times DDG was observed for the milk yield (Table 1). Cows fed diets with 40% DDG had a reduction ($P < 0.01$) in the milk yield compared with cows fed diets containing 0 or 20% DDG. There was an interaction ($P < 0.01$) of NDFf and DDG for MEC. The cows submitted to treatments with low NDFf showed a quadratic effect ($P < 0.01$), with a lower MEC with 40% DDG inclusion in the diet (Figure 2). However, in treatments with high concentrations of NDFf, we observed a decrease in the linear effect according to DDG inclusion in the diet.

There was an interaction ($P < 0.01$) of NDFf and DDG for the feed efficiency. The cows submitted to treatments with low NDFf did not show an effect on feed efficiency ($P < 0.01$) from DDG inclusion in the diet (Figure 3). However, in treatments with high concentrations of NDFf, we observed a quadratic effect ($P < 0.01$), with a lower feed efficiency for 20% DDG inclusion in the diet.

There was a linear decrease ($P = 0.02$) in MUNs with increasing DDGs in the diet. The inclusion of DDGs did not affect ($P > 0.05$) milk fat, lactose, or total solids. There was an interaction ($P < 0.01$) of NDFf and DDG for the nonfat solid yield (NFS; Figure 4), with a linear decreasing effect ($P < 0.01$) for NFS among treatments with low NDFf. However, in treatments with a high concentration of NDFf, we observed no effect ($P > 0.05$) on the NFS with an increased inclusion of DDG in the diet.

There was an interaction ($P < 0.01$) of NDFf and DDG for milk protein. The cows submitted to treatments with low NDFf showed a decreasing linear effect ($P < 0.01$)

according to DDG inclusion in the diet (Figure 5). However, in treatments with high concentrations of NDFf, we observed no reduction in milk protein.

Nitrogen Balance and Microbial Efficiency

There was an interaction ($P < 0.01$) between NDFf and DDG for N intake (Figure 6) and retained N (Figure 7). Regarding the DDG level, a reduction ($P < 0.01$) in the excretion of N in urine and milk with the inclusion of DDG was observed. We observed a quadratic effect ($P < 0.01$) for N excretion in feces for cows fed diets with DDG.

The N intake decreased ($P < 0.01$) with DDG inclusion in the diet and reduced NDFf levels. For high NDFf, no difference ($P > 0.05$) was observed for N intake, despite lower consumption when compared to diets with a lower level of NDFf (552.8 mg/kg^{0.75} for low NDFf vs. 491.3 mg/kg^{0.75} for high NDFf).

The retained N presented a deficit for all cows, except for the animals fed 0% DDG and lower levels of NDFf. There was a greater ($P < 0.01$) deficit of N with increased DDG levels and a lower NDFf concentration, but for treatments with high NDFf, no difference ($P > 0.05$) was observed.

Plasma Metabolites and Hormones

There was no interaction ($P > 0.05$) between NDFf and DDGs and plasma metabolites and hormones. The plasma glucose concentration was lower ($P = 0.02$) for cows fed 20 or 40% DDG (62.7 and 62.8 mg/dL, respectively) than for cows fed diets 0% DDG (66.3 mg/dL). Reductions in total cholesterol and urea concentrations were observed for high

DDG levels in the diet (Table 3). The dietary treatments did not affect plasma triglyceride ($P = 0.73$), IGF-1 ($P = 0.39$), or insulin ($P = 0.75$) concentrations.

Economic Analysis of the Diets

The calculated feed costs (\$/cow per day) were 3.49, 2.95, 2.08, 3.37, 3.11, and 2.24 for the low-forage 0DDG, 20DDG, and 40DDG diets and high-forage 0DDG, 20DDG, and 40DDG diets, respectively. The calculated IOFCs (\$/cow per day) were 6.33, 7.44, 6.42, 7.07, 7.06, and 6.11 for the low-forage 0DDG, 20DDG, and 40DDG diets and high-forage 0DDG, 20DDG, and 40DDG diets, respectively.

DISCUSSION

The milk yield was similar for cows fed 0 and 20% DDG; however, with the inclusion of 40% DDG, a reduction of approximately 20% was observed. The reduction in milk yields for cows fed diets with 40% DDG must be related to the reduction in DMI (Viana et al., 2021). A reduction in DMI of 12% was observed for cows fed 40% DDG relative to those fed 0 and 20% DDG inclusions. Moreover, diets with DDG had lower starch contents, at 27, 24, and 19% in the diet for 0, 20, and 40% DDG, respectively. Akins et al. (2014) observed that cows fed a normal-starch diet (27% starch of DM) produced more milk than those fed a reduced-starch diet (20% starch of DM). Fermentation of diets with greater starch concentrations produces a greater molar proportion of propionate in the rumen, contributing to an increase in the milk yields of cows fed diets with 0 and 20% DDG (Ranathunga et al., 2018). These results agree with previous studies (Mjoun et al., 2010; Ranathunga et al., 2018), which showed that lactating cows fed diets containing approximately 20% DDG on a DM basis produced similar milk yields compared to those

fed diets without DDG. However, other studies that included DDG at 20% observed an increase in milk yield compared to those fed control diets (Anderson et al., 2006; Kleinschmit et al., 2006).

Feeding highly digestible ingredients should result in improved feed efficiency, as a greater proportion of nutrients consumed become available to the animal (Bach et al., 2020). Therefore, the low digestibility of DDG combined with the highest level of NDFf may have reduced the feed efficiency. Potts et al. (2017) evaluated the contribution of digestibility to the feed efficiency and estimated that it accounts for up to 31% of the variation in residual feed intake for midlactation, depending on the starch content of the diet (14 to 30% starch).

There was a reduction in the MUN of cows fed diets containing DDG. The MUN content is correlated with the plasma urea level, which may be affected by the ammoniacal N content in the ruminal fluid and by the metabolizable protein with an inadequate profile of essential amino acids (NRC, 2001). In the present study, the milk protein decreased with increasing DDG levels in the diet and low NDFf. This result may be related to the lower acceptability by cows for diets with DDG inclusion, which may have contributed to a lower protein intake, also affecting the milk protein. In addition, the DDG used to formulate the diets showed a lower digestibility for CP (Viana et al., 2021), which may have contributed to the reduction of milk protein.

Another explanation for the reduction in milk protein may be related to dietary lysine deficiency with the inclusion of DDGs. Diets with a greater proportion of DDGs may become deficient in lysine for lactating cows. In addition, lysine is considered one of the first limitations in the synthesis of milk proteins (NRC, 2001). Morris et al. (2018) observed a reduction in the content of lysine in the diet and plasma when DDG replaced soybean meal in the diet. In addition, it has been reported that the inclusion of DDGs in

diets and an insufficient supply of lysine may decrease milk protein concentrations (Mjoun et al., 2010; Paz and Kononoff, 2014; Morris et al., 2018).

No reduction in milk protein concentration was observed with the inclusion of DDG in diets in some studies (Castillo-Lopez et al., 2014; Ramirez-Ramirez et al., 2016). However, those studies used a basal diet with a CP greater than 18.0% (DM basis); thus, the actual supply of potentially limiting AA was probably sufficient to maintain milk protein production. According to a meta-analysis conducted by Kalscheur et al. (2012), the milk protein concentration is not affected by the inclusion of DDG at up to 30% on a DM basis for lactating dairy cow diets. The reduction in N excretion in urine and in milk with the inclusion of DDGs in the diet may be related to a reduction in the N intake of cows fed DDG diets. The increase in N excretion in feces by cows fed diets with DDG may indicate less absorption of N, corroborating this result; a lower digestibility of protein was observed with the inclusion of DDG in the diets.

The lower N intake with increased DDG and lower NDF_f levels in diets may be explained by the lower acceptability by cows for DDG (Viana et al., 2021). This occurred in a similar way for the retained N—the lower was the N intake for cows fed DDG diets, the greater was the deficit observed.

Regarding the level of DDG, there was a reduction in the concentration of glucose in plasma with the inclusion of DDG in the diet, and this result may be related to a greater availability of highly degradable carbohydrates in the control diets. Highly fermentable carbohydrates promote glucose availability from gluconeogenesis, mainly affecting propionate, and consequently increase milk yields (Theurer et al., 1999). However, the values for glucose in plasma were within the range considered normal, varying between 42 and 74 mg/dL (Fraser, 1991).

The reduction in plasma urea with the inclusion of DDG is related to a lower intake and digestibility of the protein contained in the diets with DDG, and reinforcing this result, we observed a reduction in the excretion of N in milk and a lower concentration of MUN with the inclusion of DDG in diets. The reduction in plasma urea may also indicate degradable protein deficiency in the rumen (Broderick et al., 1993).

The increase in plasma concentrations and cholesterol with the inclusion of DDGs may be related to the partitioning of nutrients during lactation, in which fatty acids may be incorporated into milk fat or used to meet energy demands. The increase in the total cholesterol concentration with DDG diets is consistent with previous experiments (Mjoun et al., 2010; Ranathunga et al., 2010; Ranathunga et al., 2018), in which the increase was partially attributed to the added dietary EE. However, in this experiment, the dietary EE concentrations were similar among the diets.

The economic analysis of this study showed a numerical decrease in feed cost when DDG was included in the diets to replace corn, soybean meal, and bypass soybean meal. This decrease was attributed to replacing expensive corn starch and soybean meal and bypassing soybean meal with lower-cost DDGs. The economic advantage gained by feeding DDGs to replace corn and soybean products was reflected in the IOFC. According to IOFC analysis, we observed an economic advantage when feeding 20DDG diets compared with 0DDG and 40DDG diets. Our study reaffirms previous data demonstrating that feeding DDGS up to 20% is economically beneficial to dairy producers (Ranathunga et al., 2010 and Ranathunga 2018).

CONCLUSIONS

The distiller-dried grain concentration affects the milk yield and protein of lactating dairy cows. This study demonstrated that DDGs could be fed to lactating dairy cows at 20% DM without changing the milk yield and composition. However, the milk yield and milk protein decreased with the inclusion of 40% DM. The milk fat remained unchanged, regardless of the level of DDG in the diet. The economic analysis of the study showed a decrease in the cost of feed when DDG was included in the diets, showing an economic advantage when feeding 20% DDG in diets.

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Table 1. Performance and milk composition of cows fed different levels of distillers dried grains and forage NDF.

Item	Low NDFf ¹			High NDFf ¹			SEM ³	P-value			
	0DDG ²	20DDG ²	40DDG ²	0DDG ²	20DDG ²	40DDG ²		FDFf ¹	NDFf ¹ × DDG ²	Linear	Quadratic
Milk yield	31.18 ^a	32.26 ^a	26.34 ^c	31.11 ^a	30.42 ^{ab}	26.39 ^c	1.48	0.32	0.39	<0.01	<0.01
ECM ⁴	33.53 ^b	34.66 ^a	28.39 ^d	33.53 ^b	31.29 ^c	28.76 ^d	1.68	0.08	0.02	<0.01	<0.01
Milk 3.5% ⁵	34.46 ^{ab}	36.14 ^a	29.88 ^c	34.92 ^a	32.52 ^{ab}	29.92 ^c	1.95	0.13	0.04	<0.01	<0.01
ECM ⁴ /DMI ⁶	1.59 ^c	1.66 ^b	1.58 ^c	1.75 ^a	1.57 ^c	1.61 ^{bc}	0.07	0.32	<0.01	0.04	0.54
Milk protein	3.01 ^a	2.78 ^b	2.55 ^c	2.82 ^b	2.71 ^{bc}	2.74 ^b	0.18	0.48	<0.01	<0.01	0.38
Milk fat	4.31	4.20	4.33	4.20	4.35	4.13	0.19	0.68	0.55	0.89	0.83
Lactose	4.68	4.73	4.75	4.67	4.71	4.71	0.03	0.38	0.84	0.06	0.57
TS ⁷	13.14	12.96	12.55	12.60	12.69	12.56	0.43	0.11	0.41	0.12	0.52
NFS ⁸	8.65 ^a	8.47 ^{ab}	8.24 ^b	8.40 ^b	8.37 ^b	8.39 ^b	0.21	0.13	<0.01	<0.01	0.89
MUN ⁹	13.83 ^a	10.18 ^c	8.57 ^d	12.31 ^b	11.42 ^{bc}	10.74 ^c	1.43	0.52	0.30	<0.01	0.59

¹ NDFf = Fiber insoluble in neutral detergent from the forage; low = 21%; high = 31%;

² DDG = Percentage of distillery dried grains in the dry matter of the diet;

³ SEM = standard error of the mean;

⁴ MEC = energy-corrected milk yield;

⁵ Milk 3.5% = milk yield corrected for 3.5% fat;

⁶ DMI = Intake dry matter;

⁷ TS = total solids;

⁸ NFS = non-fat solids;

⁹ MUN = milk urea nitrogen.

Table 2. Production of microbial nitrogen, microbial efficiency, urea in the urine and nitrogen balance of cows fed experimental diets.

Item	Low NDFf ¹			High NDFf ¹			SEM ³	P-value			
	0DDG ²	20DDG ²	40DDG ²	0DDG ²	20DDG ²	40DDG ²		FDNf ¹	NDFf ¹ × DDG ²	Linear	Quadratic
Mic. nitrogen, mg/d	395.28	419.24	388.22	435.38	382.96	336.13	42.84	0.56	0.40	0.12	0.67
Mic. efficiency, mg/d	156.59	174.40	192.12	204.62	180.76	186.56	17.95	0.19	0.21	0.56	0.57
Nitrogen balance, mg/kg ^{0.75}											
Nitrogen intake, mg/d	625.72 ^a	574.23 ^b	458.39 ^d	493.29 ^{cd}	516.07 ^c	464.71 ^d	40.90	<0.01	<0.01	<0.01	0.01
Feces nitrogen, mg/d	188.36 ^c	228.67 ^{ab}	219.82 ^b	177.44 ^c	250.80 ^a	244.40 ^{ab}	16.53	0.19	0.24	<0.01	<0.01
Urine nitrogen, mg/d	278.08 ^b	263.09 ^b	204.01 ^c	323.81 ^a	257.92 ^b	206.18 ^c	27.47	0.17	0.13	<0.01	0.50
Milk nitrogen, mg/d	147.35 ^a	141.15 ^{ab}	106.64 ^c	138.39 ^{ab}	128.73 ^{ab}	113.82 ^c	13.53	0.22	0.09	<0.01	0.04
Retained nitrogen, mg/d	9.08 ^a	-59.27 ^b	-94.13 ^c	-144.98 ^d	-146.51 ^d	-100.96 ^c	0.24	<0.01	<0.01	0.14	0.81

¹ NDFf = Fiber insoluble in neutral detergent from the forage; low = 21%; high = 31%;

² DDG = Percentage of distillery dreg grains in the dry matter of the diet;

³ SEM = standard error of the mean;

⁴ N Mic = nitrogen microbial;

⁵ Efi mic = microbial efficiency.

⁶ NI = nitrogen intake;

⁷ N = nitrogen.

Table 3. Blood parameters of cows fed different levels of distillers dried grains and forage NDF.

Item	Low NDF ¹			High NDF ¹			SEM ³	P-value			
	0DDG ²	20DDG ²	40DDG ²	0DDG ²	20DDG ²	40DDG ²		FDF ¹	NDF ¹ × DDG ²	Linear	Quadratic
Glu ⁴ , mg/dL	66.86 ^a	63.26 ^b	62.40 ^b	65.66 ^a	62.15 ^b	63.13 ^b	1.94	0.64	0.74	0.01	0.14
Trig ⁵ , mg/dL	16.97	16.54	16.78	17.88	17.73	16.98	1.16	0.18	0.76	0.43	0.98
TC ⁶ , mg/dL	169.47 ^c	212.13 ^{ab}	221.37 ^a	198.12 ^b	208.64 ^{ab}	219.82 ^a	16.56	0.28	0.14	<0.01	0.27
Urea, mg/dL	40.32 ^a	37.86 ^a	32.42 ^c	40.31 ^a	36.86 ^{ab}	32.04 ^c	2.55	0.73	0.95	<0.01	0.44
IGF-1, ng/mL	319.73 ^a	319.47 ^a	226.91 ^b	223.13 ^b	237.49 ^b	252.90 ^b	53.37	0.04	0.09	0.29	0.36
Ins ⁷ , μUI/mL	2.13	1.49	2.02	1.56	1.73	1.73	0.44	0.52	0.57	0.94	0.45

¹ NDF_i = Fiber insoluble in neutral detergent from the forage; low = 21%; high = 31%;

² DDG = Percentage of distillery dried grains in the dry matter of the diet;

³ SEM = standard error of the mean;

⁴ Glu = glucose;

⁵ Trig. = triglycerides;

⁶ TC. = Total cholesterol;

⁷ Ins = insulin.

Figures

Figure 1. Experimental period scheme.

Figure 2. Interaction between NDFf and DDG for MEC

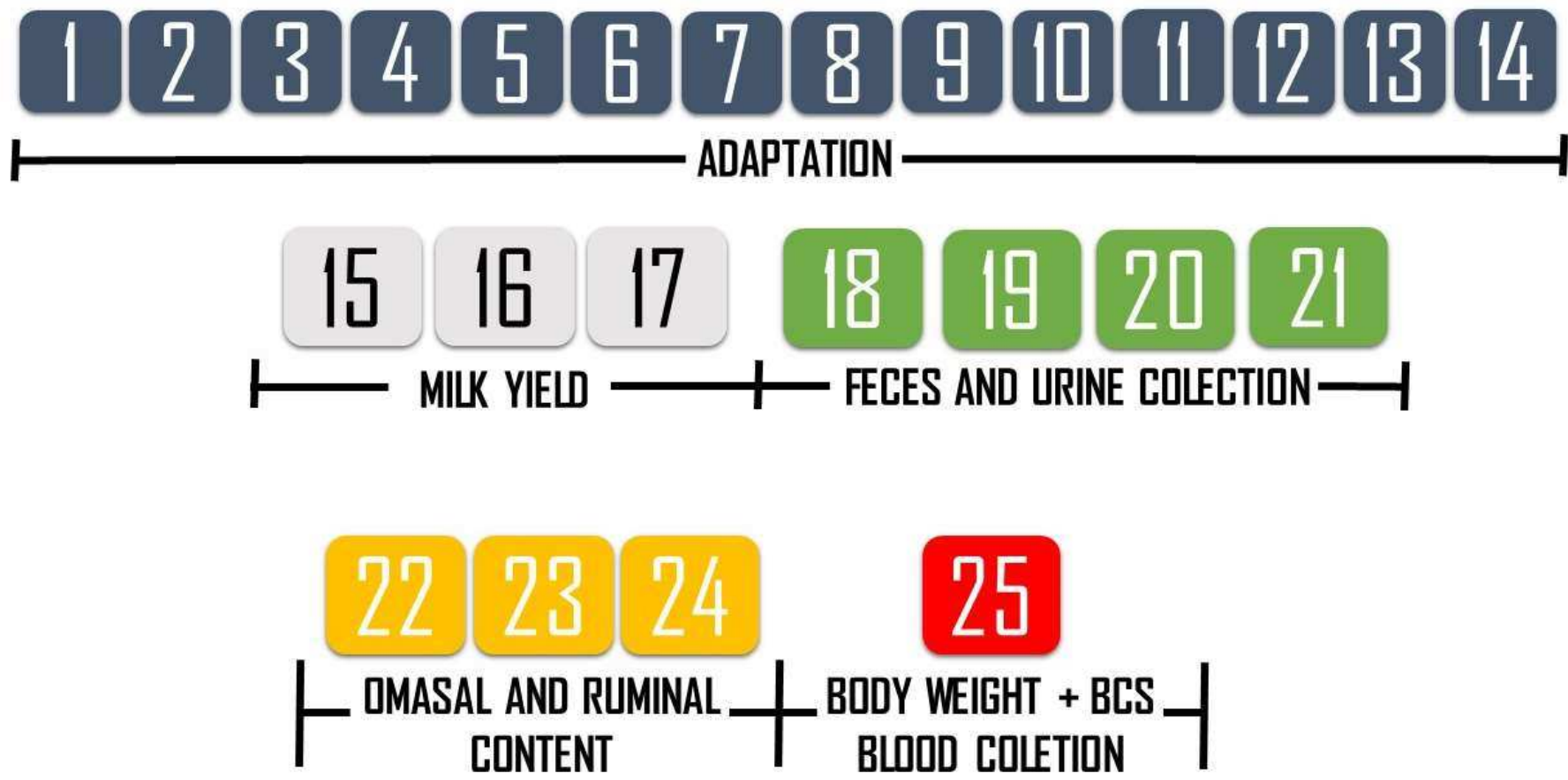
Figure 3. Interaction between NDFf and DDG for feed efficiency.

Figure 4. Interaction between NDFf and DDG for non-fat solids.

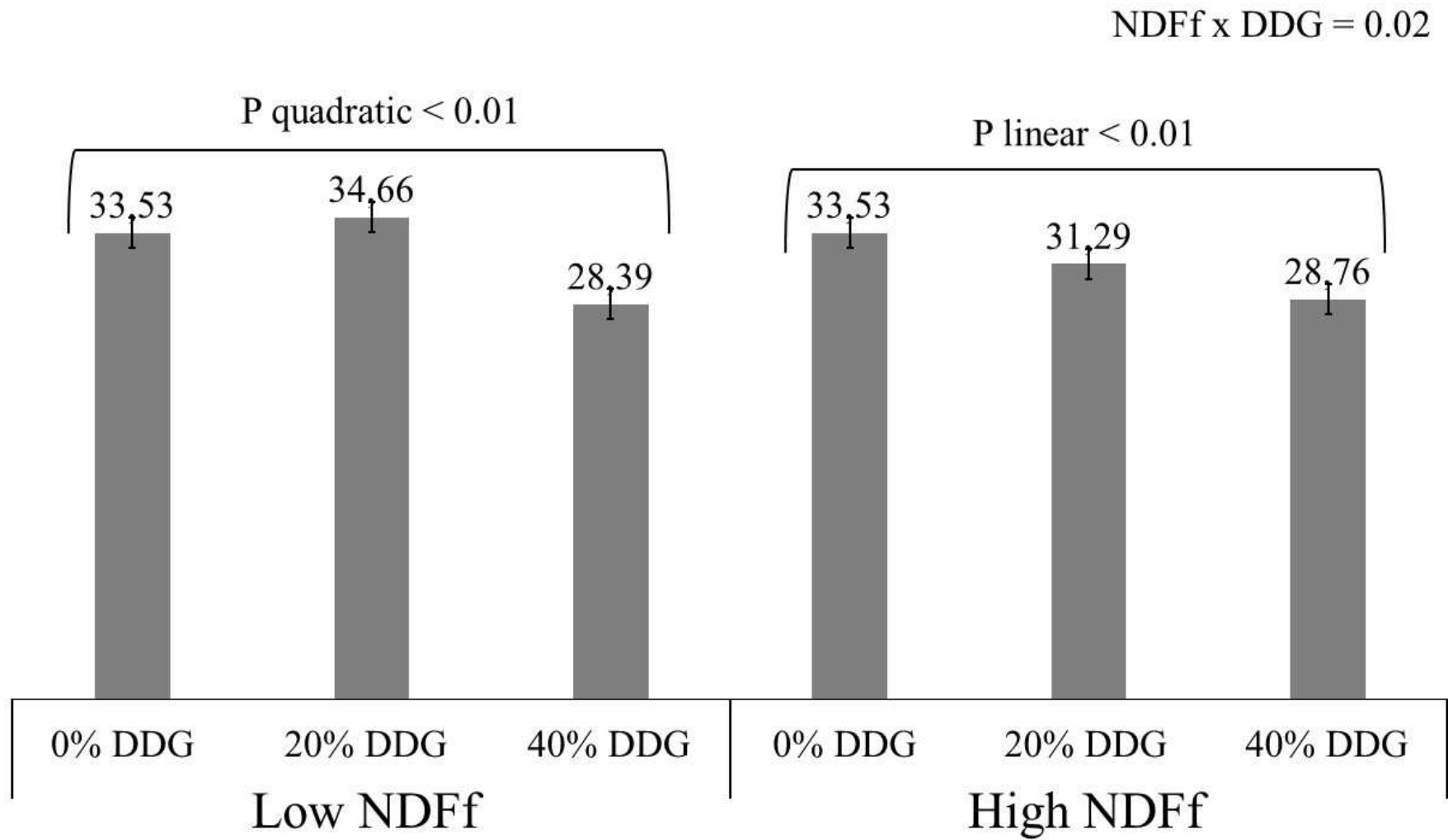
Figure 5. Interaction between NDFf and DDG for protein milk.

Figure 6. Interaction between NDFf and DDG for nitrogen intake

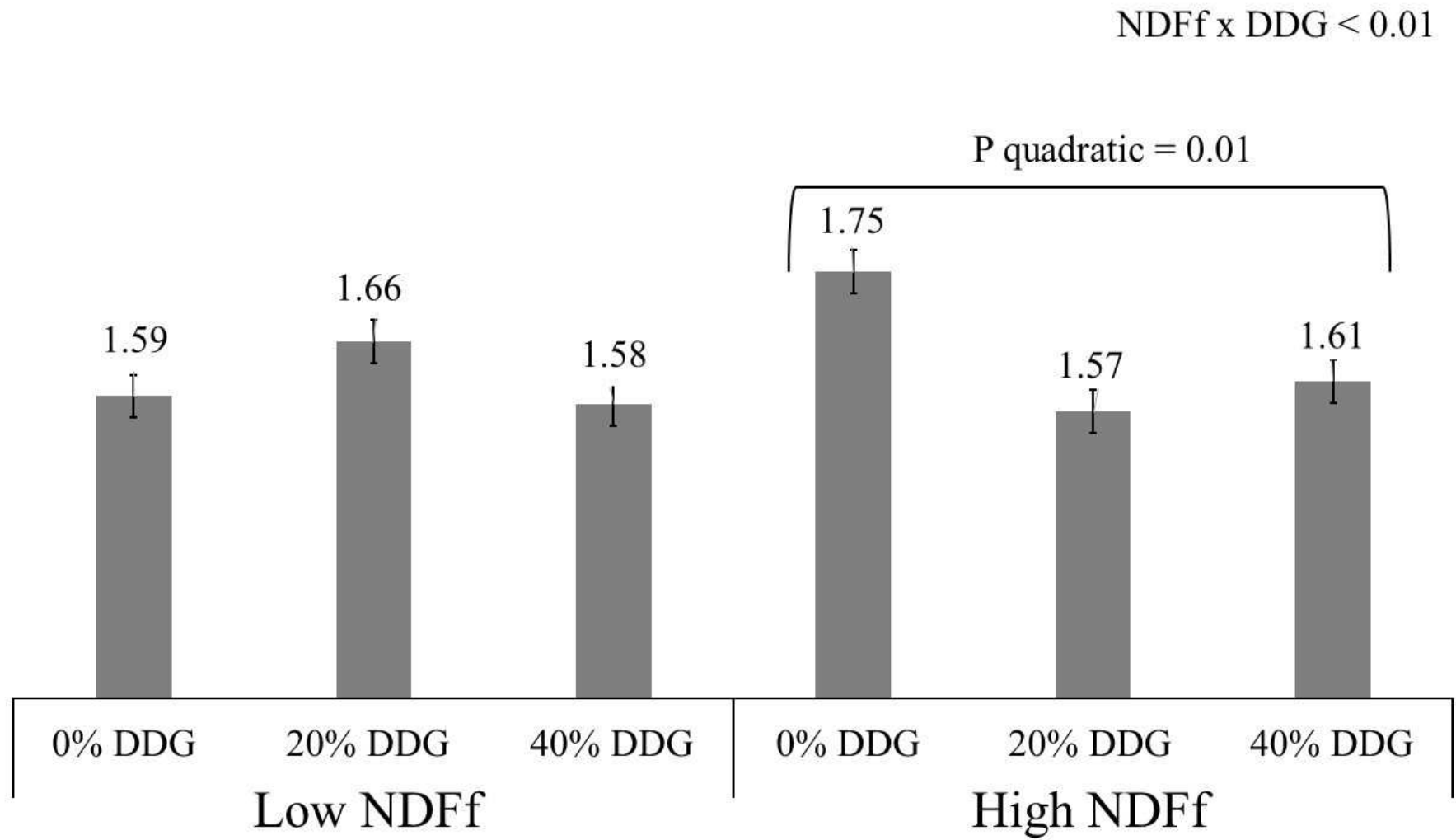
Figure 7. Retied nitrogen of cows fed different levels of DDG and NDFf.



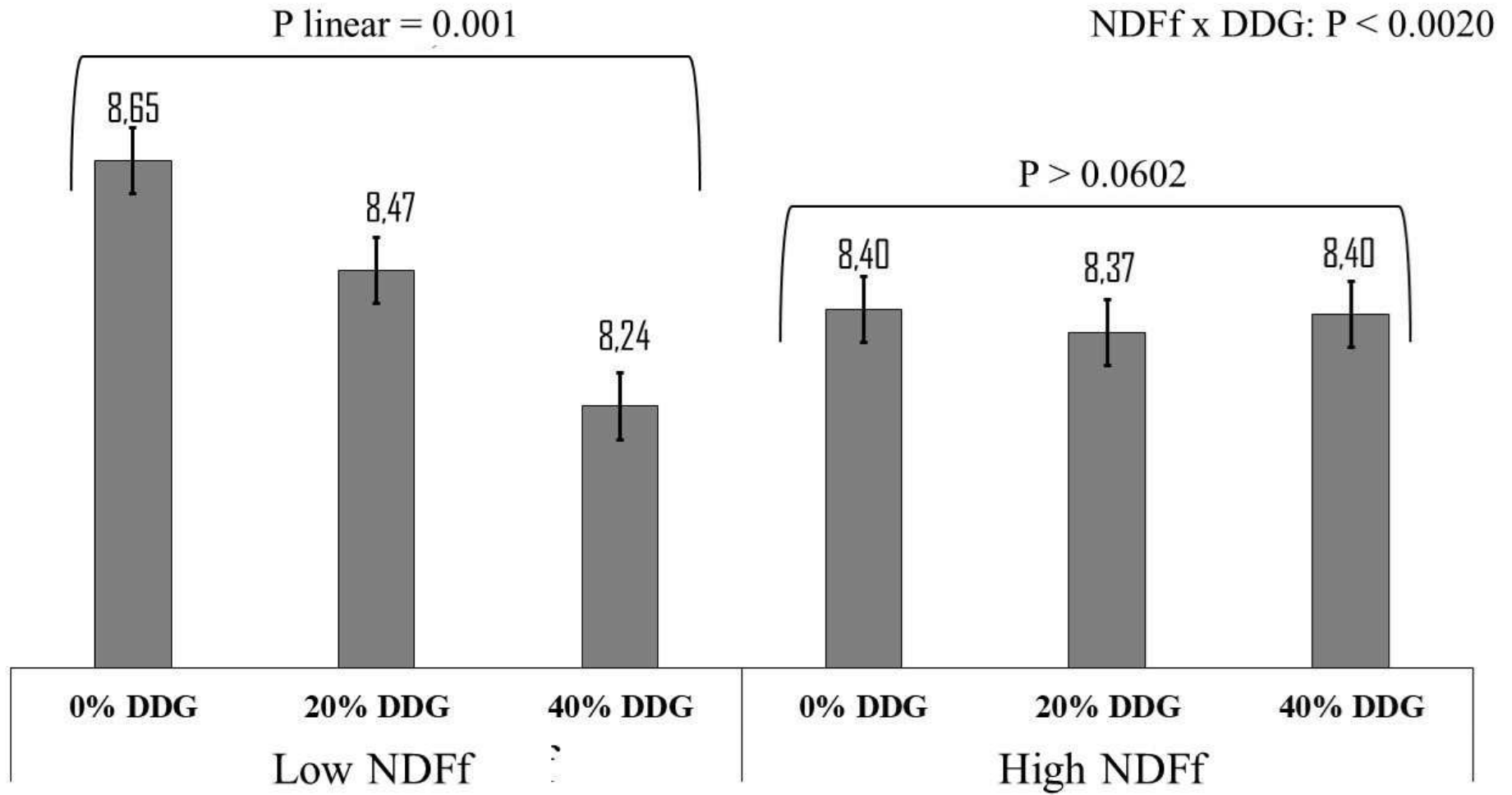
Viana, Figure 1.



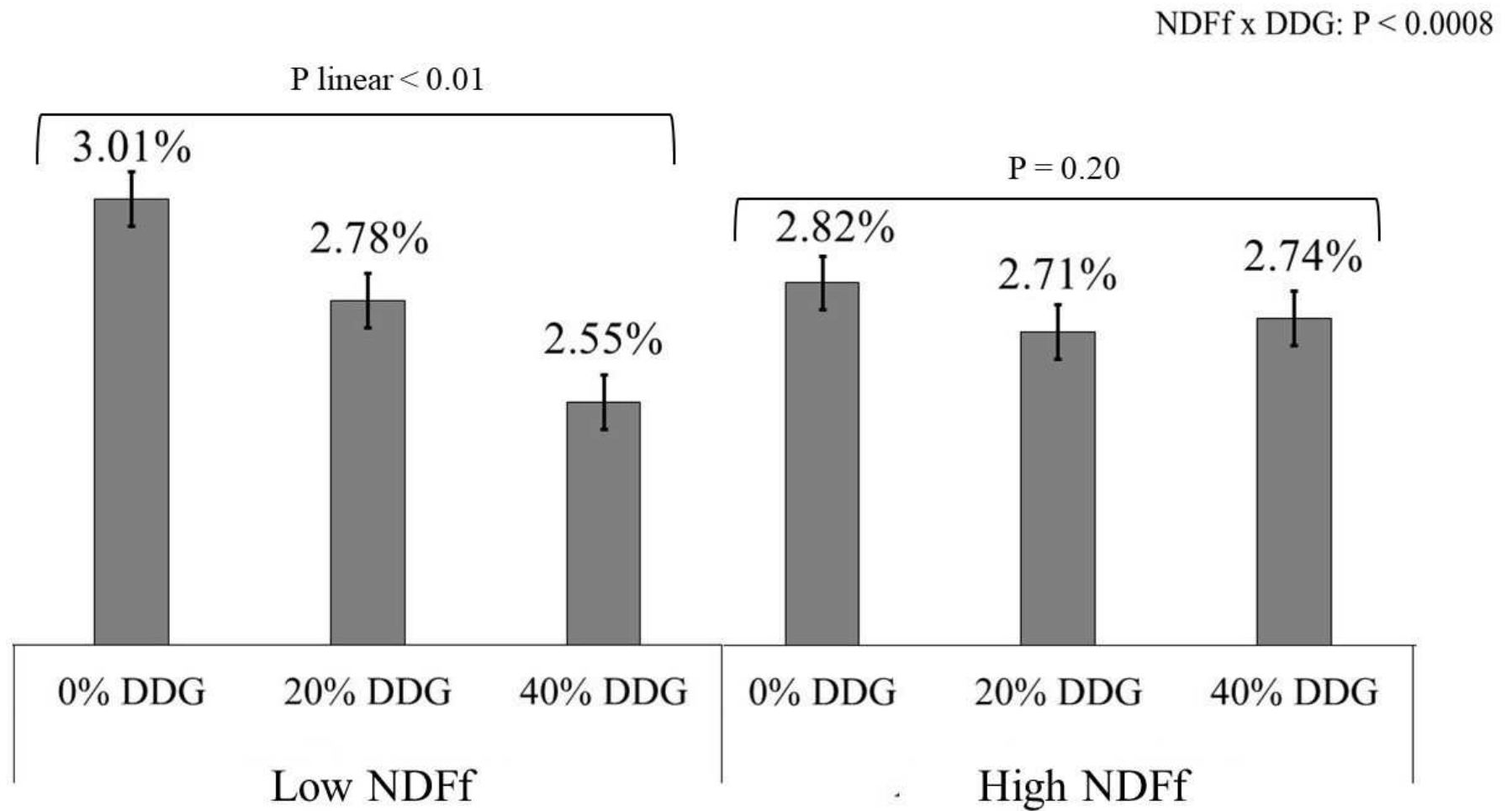
Viana, Figure 2.



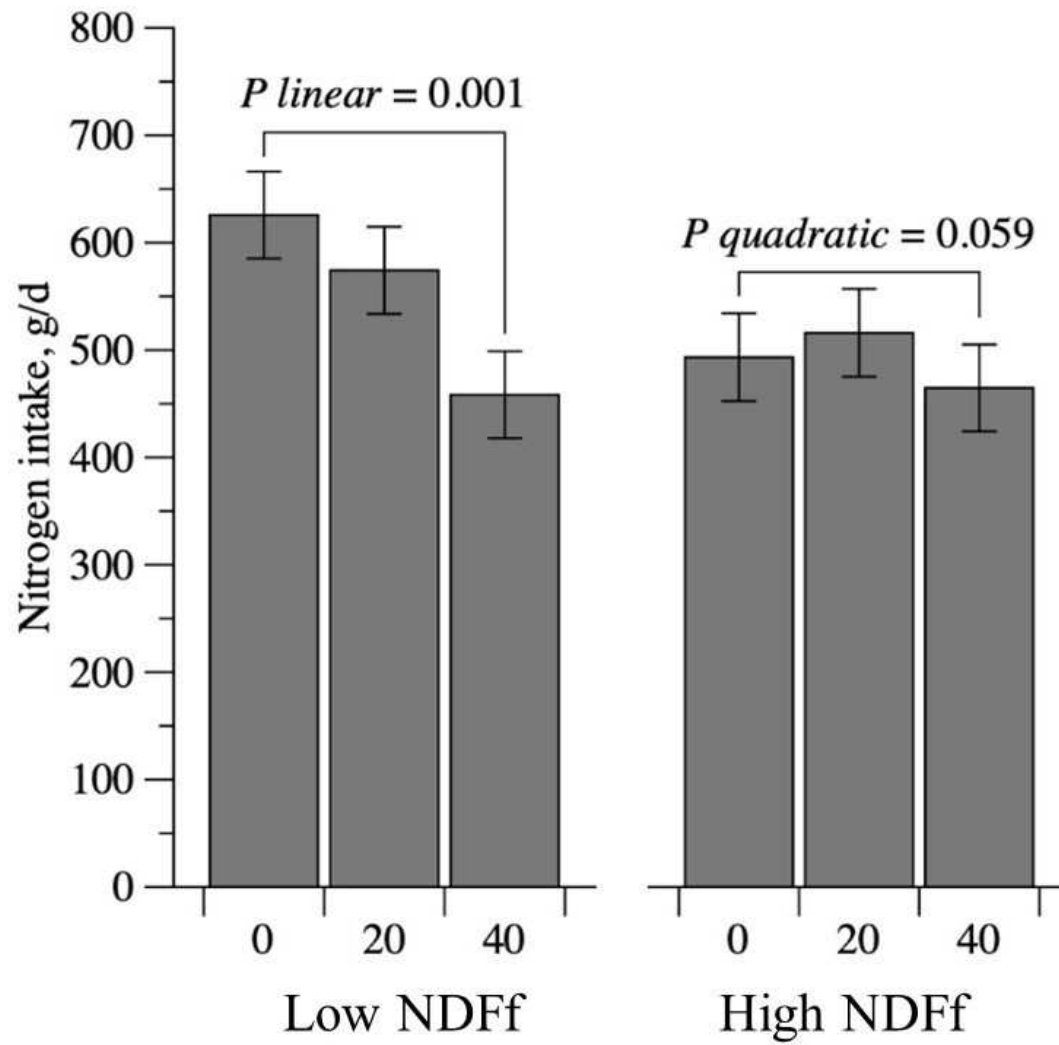
Viana, Figure 3.



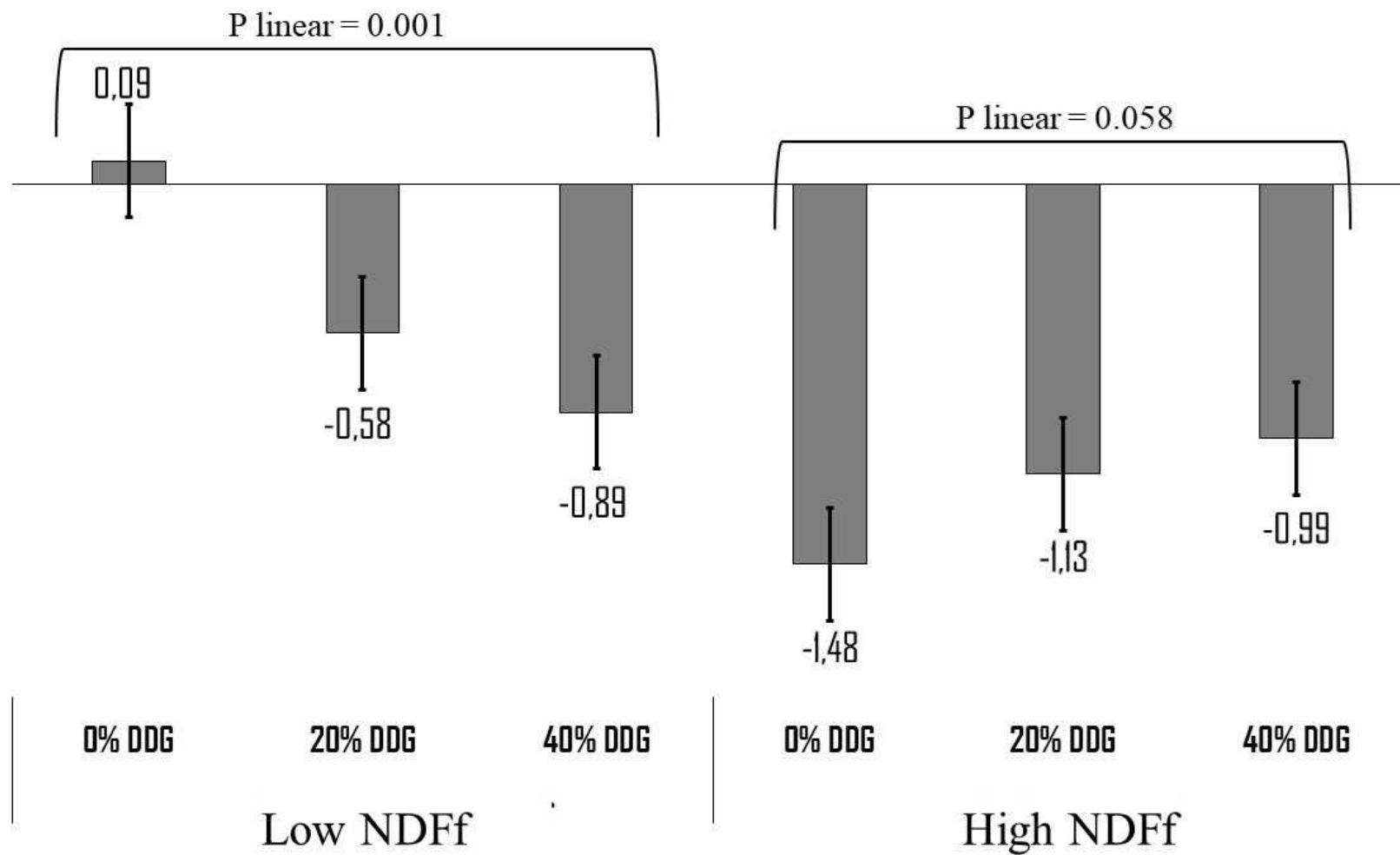
Viana, Figure 4.



Viana, Figura 5.



Viana, Figure 6.



Viana, Figure 7.