

## Temperament of New Zealand and North American Holstein cows maintained in two different feeding strategies

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#### Abstract



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Genetics is a factor that influences the dairy cows' temperament. The objective of the present study was to compare the responses to different temperament tests of New Zealand (NZ) and North American (NA) Holstein cows maintained in two different feeding strategies. A total of 120 cows from the Experimental dairy farm of the National Agricultural and Livestock Research Institute (in Spanish, INIA, Uruguay) were grouped into two Holstein strains: NZ or NA, and two feeding strategies: Grass Maximum or Grass Fixed. Milking reactivity (MR), Flight speed and Distance (FD) were assessed in two periods  $(107.5 \pm 30.3 \text{ and } 173.8 \pm 32.1 \text{ days in milk})$ . The averages of these repetitions were used in general linear models with Holstein strains (NZ vs. NA), parity (primiparous vs. multiparous), feeding system (Grass Maximum vs. Grass Fixed), and all two-factor interactions as fixed effects. For MR, there was an interaction between strains and parity. The difference between NZ and NA was observed only for multiparous cows, with higher MR in NZ (2.93  $\pm$  1.18) than in NA (2.00  $\pm$  0.90) (P=0.0004). The NZ cows had longer FD (4.2  $\pm$  1.3 m) than NA cows (3.7  $\pm$  1.1 m) (P=0.03). Feeding system did not affect the cow's reactivity. In conclusion, dairy Holstein cows' genetic strain was a factor related to MR and FD tests. In the milking parlor, multiparous NZ cows were more reactive than NA, which did not happen for the primiparous ones. The NZ cows were more reactive to humans than NA in the paddock, exhibiting longer flight distances.

Keywords: biotype, cattle, milk, personality

## Temperamento de vacas Holando de origen neozelandés y norteamericano mantenidas en dos estrategias alimentarias diferentes

#### Resumen

La genética es un factor que influye en el temperamento animal. El objetivo de este trabajo fue comparar la respuesta a tres pruebas de temperamento de vacas Holando neozelandesas (NZ) y norteamericanas (NA) bajo dos estrategias de alimentación. Ciento veinte vacas de la Unidad Experimental del Instituto Nacional de Investigación Agropecuaria (INIA, Uruguay) fueron agrupadas en una combinación de dos orígenes genéticos: NZ o NA, con dos estrategias de alimentación: Máximo pasto o Pasto fijo. La reactividad al ordeñe (RO), la velocidad de fuga y la distancia de fuga (DF) fueron



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evaluadas en dos períodos (107,5  $\pm$  30,3 y 173,8  $\pm$  32,1 días en leche), y el promedio de esas dos mediciones por animal fue utilizado en modelos lineales generales con la genética (NZ vs NA), la paridad (primíparas vs multíparas), la estrategia de alimentación (Máximo pasto vs. Pasto fijo) y sus interacciones como efectos fijos. Hubo una interacción entre origen genético y paridad para la RO. Las diferencias entre vacas NZ y NA fueron solo observadas en multíparas, presentando mayores valores las vacas NZ (2,93  $\pm$  1,18) que las NA (2,00  $\pm$  0,90) (P=0.0004). Las vacas NZ presentaron mayor DF (4,2  $\pm$  1,3 m) que vacas NA (3,7  $\pm$  1,1 m) (P=0,03). La estrategia de alimentación no afectó la respuesta a ninguna prueba. En conclusión, el origen genético de vacas lecheras Holstein influenció la reactividad a las pruebas de temperamento RO y DF. Las vacas NZ fueron más reactivas al humano y al ordeñe que las vacas NA, pero las diferencias en RO fueron solo observadas en vacas multíparas.

Palabras clave: biotipo, bovino, leche, personalidad

# Temperamento de vacas Holandesas Neozelandesas e Norte-americanas mantidas sob duas diferentes estratégias alimentares

#### Resumo

A genética é um fator que influencia o temperamento dos animais. O objetivo deste trabalho foi comparar vacas holandesas de linhagens neozelandesas (NZ) e norte-americanas (NA) quanto à reatividade avaliada por meio de três testes de temperamento. Foram avaliadas 120 vacas da Unidade Experimental do Instituto Nacional de Investigación Agropecuaria (INIA, Uruguai) de 4 grupos genéticos: NZ ou NA, mantidas sob duas estratégias alimentares: pastejo máximo ou pastejo fixo. A reatividade na ordenha (RO), a velocidade de fuga e a distância de fuga (DF) foram avaliadas em dois períodos (107,5  $\pm$  30,3 e 173,8  $\pm$  32,1 dias de lactação). A média das repetições por animal foi utilizada como variável dependente em modelos lineares gerais que incluíram a genética (NZ ou NA), ordem do parto (primíparas ou multíparas), estratégia alimentar (pastejo máximo ou pastejo fixo), e suas interações como efeitos fixos. Houve interação entre grupo genético e ordem do parto para RO. As diferenças entre vacas NZ e NA foram observadas apenas em multíparas, sendo que vacas NZ apresentaram valores maiores (2,93  $\pm$  1,18) que as vacas NA (2,00  $\pm$  0,90) (P=0,0004). As vacas NZ tiveram maior DF (4,2  $\pm$  1,3 m) que as vacas NA (3,7  $\pm$  1,1 m, P=0,03). A estratégia alimentar não teve efeito nas respostas dos testes. Conclui-se que a origem genética influenciou a reatividade nos testes de temperamento de RO e DF. As vacas NZ foram mais reativas ao homem e à ordenha do que as vacas NA, porém as diferenças na reatividade à ordenha foram observadas apenas em vacas multíparas.

Palavras-chave: biotipo, bovino, leite, personalidade

## 1. Introduction

Efficiency indicators in milk systems are affected by several factors which could be related to individual characteristics. Interindividual differences in animals' behavioral responses to different environmental circumstances are defined as temperament<sup>(1)(2)(3)</sup>. For dairy cows, temperament has been associated with productivity (milk yield, quality, and milk ability)<sup>(4)(5)(6)(7)</sup>; metabolic features<sup>(8)(9)</sup>, and behaviors such as adaptation to a novel milking system<sup>(10)(11)</sup>. However, the knowledge about what determines or modulates temperament remains limited. Genetics is a factor that influences the cows' temperament, since differences between dairy breeds have been reported<sup>(12)(13)(14)</sup>. Additionally, the differences in productivity among breeds could be, in part, explained by temperament differences<sup>(12)(15)(16)</sup>. In Uruguay, North American (NA) Holstein is the most common dairy strain (78%), but cows of New Zealand (NZ) origin (13%) have increased<sup>(17)</sup>. Previous studies have reported that NZ cows produced lower individual milk yield but higher milk component concentrations<sup>(18)</sup>, spending more time eating total mixed ration<sup>(19)</sup> and having greater energy and feed efficiency than NA cows<sup>(20)</sup>, although both had a similar economic-productive breeding index. A possible explanation for the differences in milk yield between cows of different temperament is the human-animal relationship<sup>(21)(22)</sup>. Therefore, differences in milk yield between NZ and NA could be attributed to variations in their reactivity to humans, such as



milking reactivity, across both strains. The genetic differences in temperament between strains could be due to history of pasture breeding and selection based on productivity and body size of each one <sup>(23)(24)</sup>. Additionally, the feeding system could be another factor that affects the cow's reactivity. In pasture-based management systems, human-cow interaction is typically lower than in indoor housing systems, which could result in cows under grazing systems showing greater reactivity in tests that assess fear of humans, such as increased flight distance<sup>(25)(26)(27)</sup>. According to our knowledge, studies comparing the cow's temperament of the different breeds or by different strains within the same breed are scarce. Thus, we hypothesize that the NZ cows would have greater reactivity to different temperament tests than NA ones, and cows under pasture system will show greater reactivity than cows under pasture+partial mixed ration. The objective of the present study was to compare the responses to different temperament tests of NA and NZ Holstein cows maintained in two different feeding strategies (pasture or pasture+partial mixed ration).

## 2. Materials and methods

An experiment study<sup>(18)</sup> was carried out with 120 Holstein cows over a one-lactation period at the experimental station of the National Agricultural and Livestock Research Institute (in Spanish, INIA), Colonia, Uruguay (34°20' S, 57°41' W). All procedures were approved by the Ethics Committee of INIA (file number IN-IA.2019.11). A full description of the experimental design was included in Stirling and others<sup>(18)</sup>. Four groups were derived from the combination of two feeding strategies: Grass Maximum or Grass Fixed, and two Holstein strains: NZ or NA. The Grass Maximum and Grass Fixed feeding strategies differed in the proportions of grazed pasture in the diet with the same level of concentrate per cow. The cows in Grass Fixed were fed an allowance of 1/3 pasture, 1/3 concentrate, and 1/3 silage. The silage was offered as a partial mixed ration on a concrete and dirt feedpad (4.5 m<sup>2</sup>/cow), and the total area (feeding and rest area) was 240 m<sup>2</sup> per cow. The cows in Grass Maximum had a flexible pasture allowance determined by the pasture growth rate, which was estimated weekly, offering whole-crop silage on a feedpad as a buffer in case of pasture shortage. The pasture was a mix of *Festuca arundinacea, Dactylis glomerata, Medicago sativa*, and *Lolium multiflorum*. The commercial concentrate was offered daily and individually in the milking parlor.

Thirty NA and 30 NZ cows were randomly assigned to each feeding strategy before calving, ensuring that groups were balanced for expected calving date, parity, milk yield, and milk fat content from the previous lactation. Finally, of the 60 cows in each Holstein strain cow temperament was assessed in a total of 48 NZ cows (primiparous: 12, multiparous: 36) and 50 NA cows (primiparous: 19, multiparous: 31), in the milking parlor (herringbone type) using a milking reactivity score (MR), and in the handling pen using flight speed and flight distance tests (details given below). The tests in the milking and handling pen were performed on the same day at 107.5 ± 30.3 and 173.8 ± 32.1 days in milk (two repetitions). First, the MR was assessed during afternoon milking, and then the cows were transferred to the handling pen where the other tests were performed, by the same observer. Milking reactivity was measured using a method modified from Sutherland and Huddart<sup>(28)</sup>. Each cow was scored based on the stepping and kicking behaviors using an 8-point scale during preparation for milking and attachment of the milking cluster: (1) no hind foot movement, (2) one or two slow and gentle (hoof elevated at less than 15 cm above the ground) hind limb movements, (3) three or more slow and gentle hind limb movements in inconstant rhythm (not moving during the whole procedure), (4) three or more slow and gentle hind limb constant movements (during the whole procedure), (5) vigorous hind limb movements (elevating hooves 15 cm above the ground), (6) vigorous and constant hind limb movements, (7) kicking the milker, elevating the hind hoof above hock line and directing it laterally, and (8) the cow presented high reactivity and had to have one or both hind limbs tied to enable the procedure to be performed. The observation was made by one observer positioned behind the animal in the milker pit, being able to observe both hind legs.



Flight speed (FS) was measured using a method modified from Gibbons and others<sup>(29)</sup>. The time taken by each cow to exit the squeeze chute and cover a distance of 2.7 m was recorded using a purpose-built device that measured exit speed. The unit comprised two light beams and reflectors and one readout unit. Once the cow passed through both light beams, the exit time was recorded in seconds and converted into speed, in m/s. The handler stood approximately 1 m away from the side of the crush and behind the shoulder of the animal during the test. Flight distance (FD) was measured using a method modified by Waiblinger and others<sup>(26)</sup>. The distance (in m) that cows allowed a non-familiar person to approach before expressing the first withdrawal response was recorded. The test was performed with each cow individually kept in a pen of 131 m<sup>2</sup>. After the cows exited the squeeze chute, the observer remained stationary on the opposite side of the pen entrance, approximately 8 m away from the cow, waiting for the cow to stand still before starting the test. Then, the person approached the animal slowly (one step per s), with her hands down and arms held close to the body. When the cow expressed any withdrawal reaction (i.e., the animal moved both forelimbs), the observer stopped and measured the distance to the cow's nearest front hoof using an odometer (MW40M, Stanley, USA).

Cows were weighed individually and fortnightly after the morning milking throughout lactation using a portable electronic scale (Farmquip Ltd., Hawke's Bay, New Zealand). All statistical analyses were carried out with SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Univariate analyses were performed to identify outliers and to verify the normality of residuals, and homogeneity of variances was verified with Levene's test. Considering that the day of assessment (107.5 and 173.8 days in milk) did not affect the cow's reactivity, we averaged the two repetitions by each test to obtain a single value per cow for the analyses. General linear models (GLM procedure) were used for the dependent variables FS and FD, and a generalized linear model with gamma distribution and inverse link function (GENMOD procedure) was used for MR. Fixed effects of Holstein strains (NZ and NA), parity (primiparous and multiparous), feeding system (Grass Maximum and Grass Fixed), and all two-factor interactions were included. Non-significant interactions ( $\alpha$ >0.05) were excluded from the final models. In addition, body weight average was compared between primiparous and multiparous cows by GLM procedure. Post-hoc analysis was performed using Tukey's test. The  $\alpha$  values were considered significant when ≤0.05, and the data are presented as least square means ± SEM.

## 3. Results and discussion

For MR, the main factors of strain and parity were not significant, but their interaction affected cows' reactivity (P=0.05). The difference on MR between NZ and NA was observed only for multiparous cows, with higher milking reactivity for NZ than NA (**Table 1**). The Holstein strains differed for FD (P=0.03), with longer flight distances for NZ ( $4.2 \pm 1.3 \text{ m}$ ) than for NA ( $3.7 \pm 1.1 \text{ m}$ ) cows, without parity effect. The FS was not affected by both factors studied ( $1.18 \pm 0.3 \text{ m/s}$ ; mean  $\pm$  SD). Finally, the feeding system did not affect the cow's reactivity nor their interactions with strain.

Table 1. Milking reactivity (MR) of primiparous and multiparous Holstein cows of two genetic	strains
(North American - NA, and New Zealand - NZ)	

		NZ	NA
MR (score)	Primiparous	3.40 ± 1.53Aa	3.10 ± 0.81Aa
	Multiparous	2.93 ± 1.18Aa	2.00 ± 0.90Bb

Different letters in the same column are main statistical differences between Holstein strain in the same cow parity (capital letters, p≤0.05), and between parity in the same strain (lowercase letters p≤0.05).



To our knowledge, this is the first study evaluating the relationship between temperament tests and Holstein genetic strains. The current study showed that NZ cows were more reactive to humans in both milking (MR) and paddock (FD) than NA cows. However, at milking this difference was only shown for multiparous cows. Differences for temperament among dairy breeds have been reported in previous studies<sup>(30)</sup>. For example, in a study conducted in New Zealand Jersey, cows were regarded as more "placid" during milking than Holstein cows of NZ origin<sup>(31)</sup>. In its turn, Mpisana and others<sup>(32)</sup> compared crossbred Jersey × Friesian with purebred Friesian cows in South Africa, showing that the crossbreds had greater avoidance distance but lower milking reactivity than the Friesian cows reared in the same environment. To our knowledge, this is the first study to examine temperament differences of NZ and NA breeding lines. As evidenced in our study, cows of different genetic lines cannot only differ in physical and productive parameters<sup>(18)(20)(23)(24)</sup>, but also in their behavior. The genetic selection could explain the greater flight distance and milking reactivity in NZ than in NA cows, since both lines were breeding under different environmental circumstances and with different goals. The effects of selection primarily for some traits (e.g. productivity or body size) could be related to the temperament genomic loci<sup>(30)(33)(34)</sup>. On one hand, for Holstein cows of NA origin the genetic correlation between milking temperament and milk flow was reported, as well as genetic correlations between bull EBV for milking temperament and milk flow and with various reproduction, conformation, and auxiliary traits<sup>(35)</sup>. On the other hand, the NZ cows have been selected for smaller body size<sup>(23)</sup>, which could be associated with reactive temperament<sup>(34)</sup>. Additionally, cows of lower weight or smaller sizes could be characterized as more "nervous/anxious"<sup>(32)</sup>, since smaller animals would have more room to move in the milking parlor<sup>(36)</sup>. This also might be an explanation for our findings, since our NZ cows were smaller than NA<sup>(18)</sup>. The NZ cows were also more reactive than NA cows for MR test, but this was observed only in multiparous animals. First, we could speculate that for primiparous, the novel milking environment would lead to high milking reactivity, making it difficult to observe differences between strains (NZ or NA). In fact, previous studies reported that primiparous Holstein cows had higher incidence of stepping and kicking during milking than multiparous ones<sup>(4)(6)(37)</sup>. It is interesting to highlight that for NZ cows we did not observe MR differences between primiparous and multiparous.

Based on the results, we suggest that NA cows reduce their reactivity over time, which would not be the case of NZ cows. The strain differences in MR could also be attributed to variations in body size, which only differed between multiparous cows of both strains. In primiparous, the body weight did not differ for both strains (NZ=  $450.3 \pm 13.5 \text{ Kg}$ , NA=  $505.6 \pm 51.5 \text{ Kg}$ , P= 0.09). However, in multiparous, NZ cows were lighter ( $544.0 \pm 58.5 \text{ Kg}$ ) than NA multiparous cows ( $625.4 \pm 68.1 \text{ Kg}$ ) (P<0.0001), explaining the strains differences only for multiparous cows. Finally, it is interesting to note the result that the feeding system did not affect the temperament of the cows. In this sense, not only the different interaction with humans, but also the characteristics of the feed itself in composition as well as in other behavioral parameters (such as grazing) did not greatly influence temperament in our study.

Beyond our results, future studies would be important to evaluate whether different feeding systems can affect temperament by considering other variables, such as animal density, feeding management characteristics (for example, 100% confinement and 100% pasture systems), as well as different diets, among other factors. The advantages of a herd with calm animals could be fewer human accidents and less stressed cows during milk-ing, therefore, fewer negative effects on milk production. However, this could be true in conventional milking systems, as automatic milking systems (AMS) are recommended for reactive animals<sup>(11)</sup>. In this sense, future research should determine the reactivity and productivity of both Holstein strains in other milking systems, such as the AMS. In addition, reducing the susceptibility of dairy cows to be frightened should improve their ability to adapt to various farm environments and, consequently, enhance farm economy and animal welfare.



#### 4. Conclusions

In conclusion, dairy cows' genetic strain and parity were factors related to temperament in adult Holstein cows, mainly in MR and FD tests. In the milking parlor, multiparous NZ cows were more reactive than NA cows, what did not happen for the primiparous ones, for which the variations between both strains were not evident. The NZ cows were more reactive to humans than NA cows in the paddock, exhibiting longer flight distances.

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#### **Transparency of data**

Data not available: The data set that supports the results of this study is not publicly available.

#### Author contribution statement

JTMP: Conceptualization; Formal analysis; Investigation; Project administration; Writing - original draft

ACS: Conceptualization; Methodology; Writing - review & editing

JPD: Conceptualization; Methodology; Writing - review & editing

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