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Effects of Short- and Long-Distance Road Transport and Temperament on the Magnitude of β-Endorphin Response in Limousine Bulls

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Simple Summary: The effects of short- (55 km) and long-distance (2200 km) road transport and temperament on β-endorphin (β-EP) concentrations in Limousine bulls were studied, taking into account the related effect on body weight (BW) decrease. Bulls were divided into calm and reactive subjects based on their exit velocity (EV) measurements taken one week prior to transportation. The results showed significant effects of time, temperament, and their interaction on β-endorphin concentrations. β-EP increased after long-distance transport in both groups, but more markedly in calmer bulls, returning to baseline values after 15 days. Significant effects of time were also observed for BW, which decreased after long-distance road transport and returned to baseline after 15 days in both groups.

Abstract: The purpose of this study was to evaluate the effects of short- and long-distance road transport and temperament on β-endorphin (β-EP) concentrations in 23 Limousine bulls, aged 12 ± 2 months old, taking into account the related effect on body weight (BW) decrease. Animals were transported by road from France to Sicily over a distance of 2200 km in about 33 h. Bulls were divided into calm and reactive subjects based on their exit velocity (EV) measurements taken one week prior to transportation. Blood samples were taken during four different time points: in the morning, immediately before loading in baseline conditions; after the short-distance road transport of 55 km; at their arrival in Sicily after a long-distance road transport of 2200 km; and after 15 days of stabling in finishing and fattening barns, before slaughtering. Animals were weighed before departure, at the arrival in Sicily, and after 15 days. Significant effects of time, temperament, and their interaction were observed for β-EP. It increased after long-distance transport in both groups, but more markedly in calmer bulls, returning to baseline values after 15 days. Significant effects of time were also observed for BW, which decreased after long-distance road transport and restored after 15 days in both groups. The results suggest that long-distance road transport induces a significant increase in the β-endorphin concentrations in Limousine bulls, with the greatest increase in calm subjects.

Keywords: β-endorphin; body weight; bulls; temperament; transport stress

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1. Introduction

Marketing inevitably represents a stressful event for livestock species because they are removed from their original home environment, handled, transported, confined in an unknown environment, and are often mixed with unfamiliar animals, before being transported again to their final ultimate destination [1]. These stressful events occur under common commercial practices and often induce highly variable responses that are difficult to interpret in terms of animal health, well-being, or stress [2–5]. The effects of stress during long transport are assessed using a range of behavioral, physiological, and carcass quality measures [4,6]. Stress represents the greatest aspect of animal welfare and can be assessed using many quantitative physiological variables, including behavioral changes [3]. Long-distance road transport of cattle induces psycho-physical and climatic stressors that can affect animals with different aversive intensities and duration [4,7]. Additionally, long-distance road transport triggered an evident increase in thyroid, cortisol, and catecholamine responses in cattle [8] and calves [9], with an associated increase in glucose concentrations, mainly due to glycogenolysis [10]. As a result of long-distance road transport, animals can suffer from dehydration, associated with an increase in circulating total protein, albumin, and hemoglobin and an alteration of acid-base balance [11]. Adaptive physiological responses to load time stress during the transport of cattle were described, with changes in mineral homeostasis, involving mainly calcium, magnesium, sodium, potassium, and chloride metabolism [12]. Stress can induce detrimental effects on reproductive performance [13] and on the quality of food products [4,6].

Specifically, some studies investigated the role of the circulating β-endorphin (β-EP) response to stressful situations in calves [14], showing a significant decrease following restraint and confirming its primary role in attenuating acute stress responses, rather than in prolonged or chronic stressors, as recorded in mouse models [15]. β-EP is an endogenous anti-nociceptive neuropeptide and its role was recorded in pregnant cows [16] and cattle during slaughter [17]. β-EP is involved in a wide variety of biological systems and homeostatic functions, like food intake and body weight (BW) in rodents and humans [18,19], and vitally anti-nociceptive properties due to β-endorphin-related mechanisms, as recently described in cattle subjected to halal slaughter [20]. β-EP is also involved in the endocrinological response to suckling dairy cows [8] and women, with a significant increase during suckling and reaching a peak after 20 min [21], suggesting that the increased β-EP derives from an extra-hypophyseal source. The environment also appears to influence β-EP concentrations; in fact, unfamiliar surroundings induced an increase in dairy cows [22], with a following decrease when cows were acclimated to the new surroundings [23]. In addition, the influence of animal temperament on health and performance has emerged as a key area of research in several livestock species, including bulls, heifers [24–27], and stallions [28]. What is more, previous studies have consistently demonstrated a negative association between reactivity and productive efficiency. Moreover, calm cattle exhibit higher weight gain and feed efficiency than reactive animals, as evidenced in taurine [29] and zebu breeds [30]. Also, the body weight (BW) decrease recorded in steers coming from pasture was greater than in bulls during transport, followed by a better recovery during their lairage [31]. Steers transported by road for 5, 10, and 15 h lost 4.6, 6.5, and 7.0% of their live weight, and recovery to pre-transport live weight took 5 days [32,33]. Likewise, bulls traveling for 6, 9, 12, 18, and 24 h lost 4.6, 4.5, 5.7, 6.3, and 7.5% live weight compared to baseline values, and during the 24 h recovery period, live weight returned to pre-transport levels [7].

According to the extensive literature [17,20,22,23,31–33], over the last few years, it has been hypothesized that transport length could influence circulating β-BP concentrations in bulls and that the temperament of the animals could affect these changes, with potential consequences for weight loss. Thus, the aim of the study was to evaluate the effects of short- and long-distance road transport and temperament on β-EP concentrations in Limousine bulls, taking into account the related effect on BW decrease.

2. Materials and Methods

2.1. Animals

All procedures, treatments, and animal care were in compliance with the guidelines of the Italian Minister of Health for the care and protection of animals used for scientific purposes (Council Directive 86/609/EEC), and during transport and related operations (Council Directive 91/628/EEC).

All vehicles were authorized for long transports and had additional equipment to allow the animals to be transported comfortably and still meet the dictates of the legislation (Regulation published in the EC no.1/2005). This legislation, in a nutshell, provided for road transport of 14 h for cattle with a stop of at least 1 h to care for the animals, plus another 14 h with a possible extension of the journey by another 2 h if the final point of arrival was near. Checks made at the end of the transport on the route plans showed that the transports complied with the stipulated times for both overall duration and rest.

This study was carried out on 23 clinically healthy Limousine bulls, aged 12 $\pm$ 2 months, with a mean live weight of 516 $\pm$ 35 kg, transported from Soual (France) to Sicily (Italy) over a distance of 2.200 km.

All subjects came from a small–medium farm, including multiple stalls, each of which housed 10 to 15 head, with a living space of about 5 to 6 square meters per head. The individuals enjoyed loose housing on straw bedding, with free access to the outdoor paddock, feed, and water. Here, they were reared for finishing in multiple stalls on permanent bedding and fed ad libitum with farm feed, hay, and ryegrass silage, in addition to concentrate that was administered twice daily at the rate of 1.4–1.5 kg/100 kg of live weight (LW).

The farm of origin was located about 55 km from the Soual assembly center (France) and used for animal selection before animals' transport to Sicily. The animals were separated into sections within the truck on the basis of temperament, to avoid excessive competition and fighting with each other, and by homogeneity of body weight categories. Animals were transported at an average speed of 80 km/h for 33 h on a two-axis, single-decker truck whose design conforms to the requirements of the Council Regulation 1/2005/EC (Council Regulation–EC-No. 1/2005, 2005), Animals were given a one-hour rest period every eight hours of transport, and each animal was provided with no less than 2.1 m^2 of floor area. Good-quality roads were traveled, and the experienced driver was trained in transporting livestock.

The environmental temperature and relative humidity were monitored with a hygrothermograph (ST-50; Sekonic Corporation, Tokyo, Japan), and were, respectively, equal to 18 °C and 50.8% at the time of departure, and equal to 24.5 °C and 54.5% at the time of arrival.

Upon arrival at the destination, animals were provided with ad libitum straw and beet pulp at the rate of 0.5 kg/100 kg of LW, and rationed watering for the first 24 h after their arrival. Subsequently, in addition to straw, a commercial fattening feed containing 13–14% protein was initially introduced at the rate of 0.5 kg/100 kg of LW and then gradually increased up to 1 kg/100 kg of LW with free access to troughs. Over the following days, the feed concentrate intake was gradually increased to the amount of 0.7–0.8 kg/100 kg of LW, without changing the amount of beet pulp. This feeding strategy ensured a moderate energy intake and a reduced level of highly fermentable carbohydrates to mitigate the risk of acidosis.

2.2. Temperament Classification

Young animals were classified into two temperament groups based on exit velocity (EV) measurements taken one week prior to transportation. EV is an objective parameter that measures the speed (m/s) at which calves exit a working chute, as described by Burrow et al. [34] and Curley et al. [35]. The calm group (Group I) consisted of 13 bulls with the slowest EV (1.05 ± 0.05 m/s), while the reactive group (Group II) comprised 10 bulls with the fastest EV (3.14 ± 0.22 m/s).

2.3. Sampling

Blood samples (10 mL) were collected from a jugular vein, after the containment of each animal in stalls using a rope halter by the same owner, at the following time points:

(1) At the growing–finishing farm and immediately before loading, thus representing baseline conditions (10.00–11.00).
(2) At the Soual collection center, after a short-distance road transport of 55 km lasting approximately 1 h, within a restore time of 12 h (16.00–17.00).
(3) Upon arrival in Sicily, after prolonged transport covering 2.200 km and 1 h after unloading (15.00–16.00).
(4) At the finishing farm, 15 days after transport (15.00–16.00).
(5) All bulls were individually weighed after the first and last blood sampling using a mechanical cattle scale (0.5–1000 kg) in base conditions and 15 days later, respectively.

To analyze β-endorphin concentrations, an aliquot of the blood samples (2.5 mL) was transferred into polypropylene tubes containing ethlylenediaminetetraacetic acid (EDTA) (1 mg/mL of blood) and aprotinin (500 Kallicrein Inhibitor Unit (KIU)/mL of blood, ICN Biomedicals Inc., Aurora, OH, USA) and kept at 4 °C. The samples were centrifuged at 1600× *g* for 15 min at 0 °C. Plasma samples were separated and stored at −80 °C until analysis. Peptides were extracted from plasma samples with 1% trifluoroacetic acid (HPLC grade) and eluded with 60% acetonitrile (high-performance liquid chromatography (HPLC) grade) in 1% trifluoroacetic acid. Plasma β-endorphin concentrations were measured in duplicates using a commercial radioimmunoassay (RIA) kit (Peninsula Lab. Inc., Belmont, CA, USA) for β-endorphin, which has a 100% cross-reactivity. The hormone assay used a range for the amount of β-endorphin detected of 1 to 128 pg/100 mL (3–371 pmol/L). The intra- and inter-assay coefficients of variation (CVs) were 7% and 15%, respectively.

2.4. Statistical Analysis

Data, expressed as mean values ± standard deviation, were tested for normality using the Shapiro–Wilk test. All data were normally distributed ($p > 0.05$), allowing for statistical analysis. The values obtained were also tested for normality using the Kolmogorov–Smirnov test. A general linear model (GLM) for repeated measures was applied to assess the significant effects of the experimental conditions (road transport and temperament group) on the studied parameters. When significant differences were found ($p < 0.05$), Bonferroni's post hoc comparison was applied. A *t*-test was applied to assess the significant effects of the recovery conditions (after 15 days) on the studied parameters (β-EP and BW). The delta percentage (Δ%) of circulating β-endorphin concentration (pg/mL) in total and calm and reactive bulls at different post-transport sampling points was also calculated. Statistical analysis was performed using the STATISTICA software package (STATISTICA 7, Stat Software Inc., Tulsa, OK, USA).

3. Results

The results for β-EP changes along transport for all bulls, and for the calm and reactive groups, are presented in Figure 1a,b, respectively.

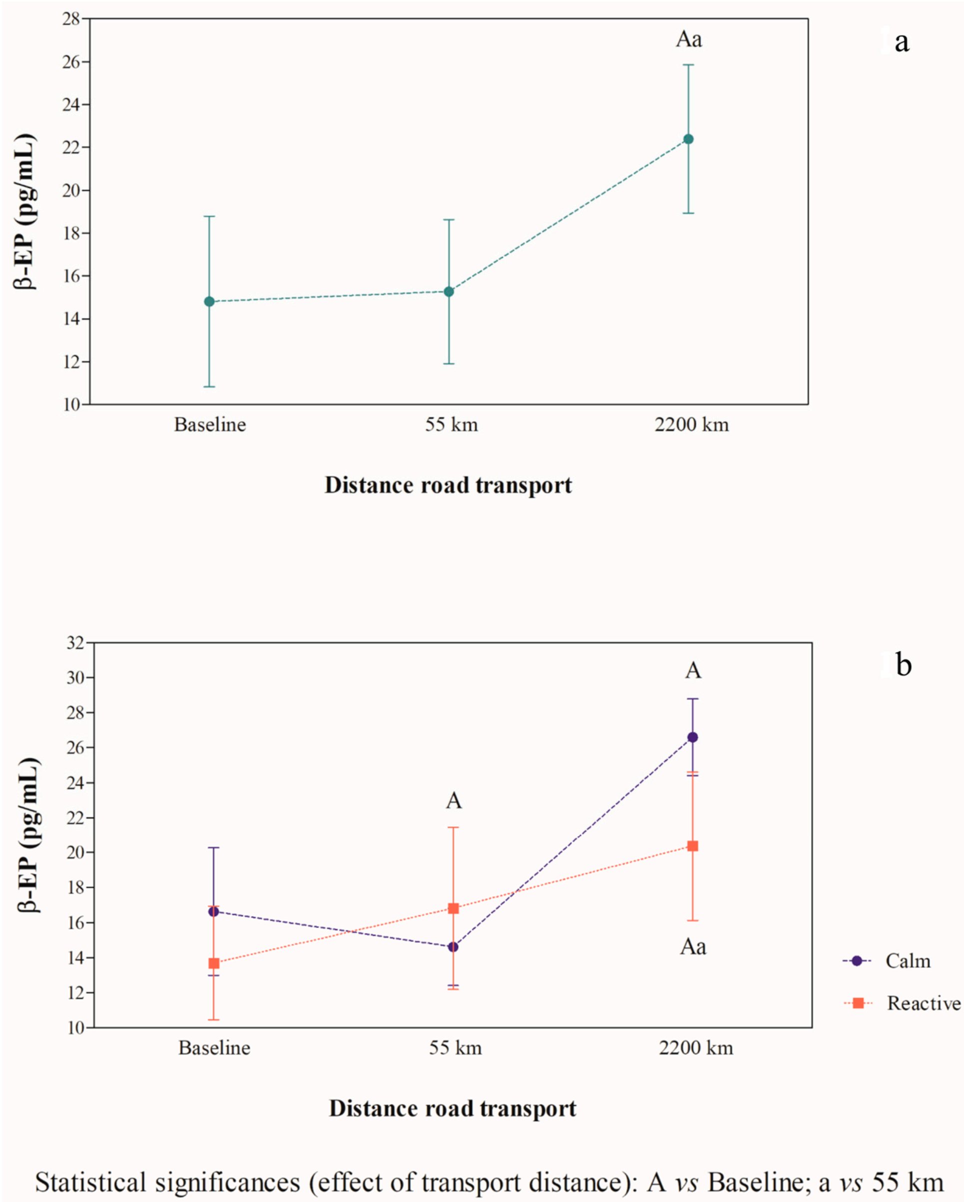

Figure 1. Circulating β-endorphin concentrations (pg/mL) of Limousine bulls (all—(**a**) and divided according to their temperament—(**b**)) at different sampling points.

Compared to baseline values, total bulls showed significant increases both after 55 km ($p < 0.0001$) and after 2200 km ($p < 0.0001$) (Figure 1a).

The results of the models indicate an interaction between long-distance road transport and β-EP concentrations in the calm and reactive groups ($p = 0.001$).

Compared to baseline values, reactive bulls showed significant increases both after 55 km ($p < 0.001$) and after 2200 km ($p = 0.001$). Compared to short-distance transport, β-EP showed greater values after long-distance transport ($p < 0.001$).

Compared to baseline values, calm bulls showed an evident peak after 2200 km ($p < 0.001$). Compared to short-distance transport, β-EP showed greater values after long-distance transport ($p < 0.001$). Greater β-EP values were observed in calm compared to reactive bulls after long-distance transport ($p < 0.01$) (Figure 1b).

Compared to baseline values, at 15 days after arrival, β-EP values returned to the superimposed range, both in total (Figure 2a) and in the two groups, with greater β-EP values in calm compared to reactive bulls ($p < 0.02$) (Figure 2b).

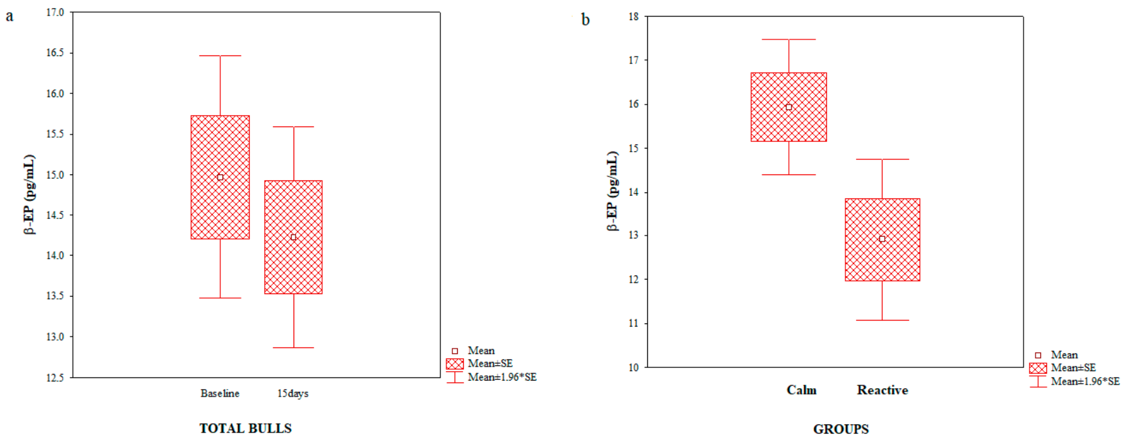

Figure 2. Circulating β-endorphin concentrations (pg/mL) of total Limousine bulls (**a**) and divided according to their temperament (**b**) at baseline and 15-day sampling points.

Specifically, β-EP concentrations of total subjects increased slightly after short-distance transport (+6%) and significantly after long-distance transport (+54%), returning approximately to baseline-like values 15 days later (−5%) (Table 1).

Table 1. Δ% of circulating β-endorphin concentration (pg/mL) in total and calm and reactive bulls at different post-transport sampling points.

Sampling Time	55 km	2200 km	15 Days
Calm	−12%	+60%	−4%
Reactive	+23%	+49%	−6%
All bulls	+6%	+54%	−5%

The results of the models for body weight (BW) changes for all bulls, as well as for calm and reactive groups, are presented in Table 2.

Table 2. Mean and standard deviation of body weight (kg) in total and calm and reactive bulls at different sampling points.

Sampling Time	Basal	Arrival	15 Days
Calm	530.40 ± 39.71 ^{A}	488.20 ± 20.48 ^{B}	533.30 ± 22.90 ^{A}
Reactive	501.28 ± 28.04 ^{AB}	447.46 ± 18.85 ^{C}	504.38 ± 25.70 ^{AB}
All bulls	513.94 ± 35.94 ^{A}	465.17 ± 28.14 ^{B}	516.96 ± 28.10 ^{A}

Different letters indicate significant differences in the mean values according to the Tukey–Kramer test.

Significant differences in BW decrease were observed across time points (baseline, arrival after 2200 km, and 15 post-transport days; $p < 0.0001$) in total animals and between temperament groups. Calm bulls exhibited a BW decrease of 6–8.5%, while reactive bulls experienced a greater decrease of 10–12.3% ($p = 0.0113$), though the differences were not statistically significant (Table 2). Transport significantly affected animals' BW ($p < 0.0001$) in a similar manner in both groups.

4. Discussion

The average β-EP concentrations recorded in the present study are in line with data previously observed in cattle [34,35], heifers [24], and buffaloes [25]. Other studies carried out on bulls, [36–49], stallions [28,41], and sheep [42] showed that both short- and long-distance road transport could differently influence the magnitude and duration of β-EP responses to stress.

Collectively, the findings reported in this paper suggest that there is a link between β-EP, temperament, and BW decrease in transported bulls. Due to these interactions, our findings demonstrate that long-distance road transport induced superimposed β-EP increases in both calm and reactive bulls, with a greater β-EP value in calm than reactive bulls after long-distance, probably stimulating adenohypophysis activity and mitigating the perception of long-lasting stressors. Nevertheless, β-EP concentrations recorded after short- and long-distance road transport remained within the physiological range reported for this species, corroborating the hypothesis that its increase could induce a defensive and adaptive response; what is more, precisely for this reason, β-EP could therefore be acceptable from the viewpoint of animal welfare. Specifically, compared to baseline values, the percentage of increase in β-EP concentrations in all subjects was 6% and 54% after short- and long-distance road transport, respectively, reflecting the onset and persistence of an adaptive mechanism to stress (habituation) rather than a direct indicator of a lower stress degree during normal management practices.

These results confirm that, given its role in mitigating stressful stimuli perception, β-EP is a valuable physiological indicator of animal welfare, expressing both physical and mental health and well-being of animals in adverse environmental or management systems [43].

While other stress-related parameters (heart rate, eye temperature, cortisol levels) are generally strongly correlated with behavioral traits [44,45], our results showed the effect of temperament on β-EP concentrations, with greater average β-EP concentrations after long-distance transport in calmer bulls than reactive ones, as shown by the interaction between these variables.

Several studies have related reactive temperament to poorer productive and reproductive performance in cattle, showing recently that reactive bulls exhibit delayed sexual development and poorer performance in body development compared to calm bulls [46].

However, despite the low interaction observed between temperament and BW loss after transport, the data obtained confirm what was recorded in cattle with greater exit velocities that showed a reduced carcass weight [47]. Therefore, based on the knowledge that many factors can contribute to temperament, including breed, gender, age, previous handling, and genetics [7,44–48], it has been well demonstrated in cattle [29] that heritability of chute score and flight speed scores were affected by breed, with Herefords having the greatest and Limousine the lowest heritability estimate for both measurements. Given that all animals were homogeneous in sex, age, and live weight, it is possible to presume that this absence of interaction could be ascribed precisely to the Limousine breed.

Nevertheless, the greater BW decrease in reactive bulls than calm after long-distance road transport is in accordance with data recorded in reactive cattle [49], which have to expend more energy to maintain their state of excitement; likewise, animals with this behavioral profile remain constantly on alert [50]. Moreover, other authors have attributed the poor weight gain performance observed in reactive animals to lower feed conversion efficiency [51] and lower dry matter intake [47]; however, these productive parameters were not evaluated in this study.

In addition, different housing systems, such as unfamiliar environments or social regrouping (French growing–finishing farm and Sicilian finishing farm) did not elicit the changes in the fluctuation of β-EP of bulls, consistent with findings in dairy cows housed in conventional finishing farming or collection animal stations [52]. Moreover, it is known that characteristics such as previous experience, genetics (temperament), age, social relationships, and human–animal interactions can modify and differentiate the stress response from one individual to another [48,53,54], interacting in a complex way to modulate the fearful response of animals also during handling or transport. Different stressors are perceived differently by different individuals, and the magnitude of the stress response depends on this perception [55].

Specifically, in our experimental conditions, bulls had no previous transport experience, supporting the influence of inexperience and individual differences in temperament in also modulating the opioid response to long-distance road transport, as previously recorded for functional, hematological, hypothalamic–hypophysis–adrenal axis, and thyroid responses of Limousine bulls [39,40].

Within 15 days, β-EP concentrations returned to baseline, likely due to physiological adaptation to the new environment or negative feedback mechanisms on the adenohypophysis that followed the increased β-EP concentrations recorded after long-distance road transport. Therefore, β-EP changes observed in bulls confirm that different impacts of handling during pre-transport (i.e., collection of animals, weighing, loading), transport itself, and post-transport handling (i.e., lairage time) have a significant effect on animals' stress-coping strategies, as previously recorded in heifers, bulls, and steers after long-distance road transport [31]. Moreover, this confirmation is further corroborated by calm bulls that exhibited a biphasic pattern, in contrast with reactive bulls that showed a gradual increase in β-EP values, and with β-EP concentrations returning to baseline values in total bulls recovered after 15 d. This means that if the disturbance lasts a short period, this β-EP response is extremely adaptive and the welfare of the animal is not impaired.

5. Conclusions

In the past decade, interest has grown in the study of temperament's effect on the performance of growing calves, showing that animals with faster exit velocities and greater temperament scores were less productive. Considering its impact on productive and reproductive efficiency, an animal's temperament represents a critical trait considered in most breeding programs. Collectively, the results obtained showed a link between β-EP, long-distance road transport, and BW decrease in bulls, represented by greater β-EP values in calm than reactive subjects. This is probably an expression of greater adenohypophysis activity stimulation to mitigate the perception of long-lasting stressors. The maintenance of ensured welfare in farm animals depends on an appropriate assessment of their welfare state in order to avoid inappropriate management and to maintain good transport and restore conditions, and β-EP can be a valuable marker of animal welfare. The use of physiological and behavioral indicators is encouraged, because they are quantitative and not subjective approaches for a more comprehensive evaluation of the impact of transport on animals' welfare.

This study highlights the physiological role of β-EP in strenuous transport response, interpreting its increase as a defensive and adaptive mechanism to stress, rather than a direct indicator of a lower stress degree, preparing the animal to be able to cope with the situation in several cases, including normal management practices.

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Institutional Review Board Statement: All analyses were carried out on animals to be slaughtered for human consumption, in accordance with the Italian and European reference legislation, which guarantees animal protection at slaughter (EU Directive 93/119/EC). The Committee for the Care and Use of Animals of Messina University declared that the proposed study could not be considered an animal experimentation and thus did not need ethical approval according to Italian law.

Informed Consent Statement: Informed consent from the bulls' owners was obtained for each animal that participated in this study.

Data Availability Statement: The data that support this study will be shared upon reasonable request to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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