



Bovine Research

Changes in behavior and plasma metabolites after tryptophan supplementation in steers



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ABSTRACT

Tryptophan (Trp) is an amino acid used as precursor for several molecules in the body, including serotonin. Serotonin is involved in a broad range of physiological and behavioral functions, including stress response. Because serotonin cannot cross the blood-brain barrier, supply of Trp to the brain could be used to elevate serotonin synthesis and release. Evidence in monogastric species indicates that Trp supplementation may increase circulating serotonin and reduces stress response, but there is conflicting evidence in the literature about degradation of Trp in the rumen. Some authors indicated that Trp is degraded slowly in the rumen and that it may remain available to be absorbed, whereas others observed that it is quickly degraded by rumen bacteria. Thus, the goal of the experiment was to test if supplementation of free L-Trp in steers may increase plasma Trp and serotonin, thus changing behavior and reducing circulating concentrations of hormones that are related to stress response. Four hybrid steers were used in a Latin square design with supplementation of L-Trp at 0, 50, 100, and 150 mg/kg BW/d for one week and three weeks as washout period. Blood samples were analyzed for amino acids, adrenocorticotrophic hormone, adrenaline, cortisol, serotonin, and glucose. Videos were used to evaluate posture and activities every 10 minutes. Results indicated no effect of diet or diet by day interaction for most of the evaluated variables and activities. The only difference detected was a diet effect on time spent inactive. Steers receiving 150 mg Trp/kg BW spent more time inactive than steers in the control (69.51 ± 2.01 vs. 60.9 ± 1.92 ; $P = 0.0305$). We concluded that it was not possible to raise plasma Trp in steers using up to 150 mg/kg BW/d free Trp and that higher doses might be needed to increase plasma Trp and Trp:large neutral amino acid ratio, and to induce meaningful behavioral changes in steers.

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Introduction

Tryptophan (Trp) is an essential amino acid that is used for protein synthesis and as precursor for several active molecules in the body (Wu, 2009). About 95% of Trp that is not used for protein synthesis is degraded by the kynurenine pathway and hence is oxidized completely to carbon dioxide and water by way of acetyl CoA (Bender, 1982). The first and limiting step of the kynurenine pathway is conversion of tryptophan to N¹-formylkynurenine, primarily by the enzyme Trp 2,3-dioxygenase (TDO) in the liver. The flux of Trp down the kynurenine pathway is regulated mostly by

plasma free Trp and secondarily by TDO activity, which has a short half-life (about 2 hours) and rapid inducibility (Badawy, 2017).

There are three other pathways of degradation of Trp. These pathways might metabolize a small fraction of total Trp but some of their products have key physiological roles. One of these smaller pathways is the 5-hydroxyindole pathway, leading to the synthesis of the neurotransmitter serotonin (Bender, 1982). Serotonin is involved in a broad range of physiological and behavioral functions, including stress response. Serotonin cannot cross the blood-brain barrier, but because key regulatory enzymes in its synthesis are not completely saturated with their substrates, increasing supply of Trp to the brain elevates serotonin synthesis and release (Markus, 2008). After an oral administration of Trp to rats, serotonin levels showed a similar increasing pattern in the brain and blood, so platelet serotonin concentration has been suggested as a peripheral index of brain serotonin (Collins et al., 2013).

All the large neutral amino acids (LNAAs; i.e., Trp, phenylalanine, tyrosine, leucine, isoleucine, and valine) compete for the same

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carrier at the blood-brain barrier, so the effect of plasma Trp in serotonin synthesis depends not only on Trp concentration but on the ratio of plasma Trp to the sum of the other competing LNAAs (Markus, 2008).

Evidence in animals supports the idea that a chronic increase in brain serotonin reduces stress response. After serotonin was infused into the lateral ventricle of unanesthetized sheep, a reduced response of the hypothalamic-pituitary axis to 15 min of restraint stress was observed (Frey and Moberg, 1981). Other authors (Koopmans et al., 2006) observed that supplementing Trp (5 g/kg) to piglets increased hypothalamic serotonin, reduced salivary cortisol, and reduced physical activity after 10 d of supplementation. There is also similar evidence in trout (Höglund et al., 2007) and humans (Markus et al., 2000).

Furthermore, Bruschetta et al. (2010) observed strong negative correlations between plasma serotonin and glucose, plasma serotonin and hematocrit, and between plasma Trp and cortisol in dairy cows. As glucose, hematocrit, and cortisol normally increase during stress, these strong negative correlations with plasma serotonin and Trp in cows suggest a similar pattern of changes in brain and blood serotonin, which is consistent with the observations of Collins et al. (2013) in rats.

Increasing brain and plasma serotonin in ruminants by Trp supplementation requires low Trp degradation in the rumen, but there is conflicting evidence in the literature about its ruminal degradation rates. Lewis and Emery (1962) divided amino acids into three groups and considered Trp to be in the lowest deamination rate group. Other studies showed that rumen microorganisms metabolize Trp slowly, so it may remain available to be absorbed (Candlish et al., 1970), and that compared to other amino acids, Trp “is metabolized more slowly by rumen microorganisms” (Mohammed et al., 2003).

In contrast, other authors (Ma et al., 2012) declared that if Trp is not protected from rumen microorganisms, it is quickly degraded by rumen bacteria. Similarly, rumen-protected Trp was used for supplementing heifers and dairy cows (Kollmann et al., 2008), assuming that free Trp would be degraded and used for *de novo* protein synthesis by rumen bacteria.

Previously, we observed low rumen degradation of purified L-Trp *in vitro* (<18% after 48 h; Marín et al., 2013). Therefore, the goal of this experiment was to test if supplementation of free L-Trp in growing steers under confinement may increase plasma Trp and serotonin, thus changing behavior and reducing circulating concentrations of hormones that are related to stress response.

Materials and methods

Animals, diets, and experimental design

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research and were approved by the institutional Bioethics Committee of Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, and CONICYT, Chile. Four hybrid steers (Holstein × Montbeliarde) of 311 ± 15 kg of body weight (BW \pm SD) were used in a Latin square design with four diets and four periods. Thus, every steer was fed once with each of the experimental diets, and the four experimental diets were fed in each of the experimental periods. All steers were kept in a single pen. The animals received a base diet with alfalfa (*Medicago sativa* L.) hay, corn (*Zea mays* L.) grain, and wheat (*Triticum aestivum* L.) straw. The animals had *ad libitum* access to a block of mineral salts and fresh water. The base diet was formulated to meet nutrient requirements for an average daily gain of 150 g/d based on NRC (2000) and was provided to the animals twice per day.

Treatments were supplementation of L-Trp (Ajinomoto Eurolysine SAS) at 0, 50, 100, and 150 mg of L-Trp/kg BW/d. Tryptophan concentrations were selected based on literature from other mammalian species (Firk and Markus, 2009; Koopmans et al., 2009; Poletto et al., 2010) and considering *in vitro* rumen availability as measured by Marín et al. (2013). Tryptophan was given in a single dose with the morning meal, by suspending it in warm water in a long-neck bottle. The animals were forced to drink the suspension by holding their heads in a slightly raised position with a hand-held nose lead. The bottle was rinsed twice and each rinse was given to drink to the steer as described.

Supplementation was provided for one week, and there was a three-week washout after each supplementation period. Three weeks was used as a washout because in pigs, the hypothalamic serotonin concentration peaked after 5 days of Trp supplementation, and after 10 days, its concentration returned to basal levels (Adeola and Ball, 1992). Furthermore, to balance any carryover effect in the Latin square design, every treatment followed every other treatment an equal number of times (Kutner et al., 2004).

Behavior analysis

A closed circuit of television with daylight and infrared vision was used to record videos for behavior evaluation. A one-minute video was recorded every 10 minutes during days –1, 0, 2, 5, and 7 of L-Trp supplementation. The first 5 to 10 seconds of each video was used to identify postures and activities as described in Table 1. Counts of standing and lying postures and drinking, feeding, eliminating, interacting, ruminating, and inactive observations were expressed as percentage of total observations recorded. Activities were considered mutually exclusive among them, but both activities and postures were observed and recorded at the same time. Thus, there was one posture and one activity recorded for each observation.

Blood sampling and analysis

Blood samples (20 mL) were taken before the afternoon meal and eight hours after the L-Trp dose, as the maximum concentration of circulating Trp was reached between 8 and 12 h after a single dose of L-Trp into the rumen of heifers (Abe et al., 1991). Blood samples were taken by coccygeal venipuncture at days 0, 2, 5, and 7 in Vacutainer Blood Collection tubes (BD, Franklin Lakes, NJ), using lithium heparin for plasma (158 USP units) and no clot activator for serum.

Plasma amino acids were measured by HPLC at Laboratorio Barnafi Krause (Santiago, Chile) using a Biochrom Amino Acid Analyzer, after deproteinization in 2.5% 5-sulfosalicylic acid. An

Table 1

Ethogram used for estimation of behavior and postures in steers (modified from Poletto et al., 2010)

Behaviors
<ul style="list-style-type: none"> • Inactive: Physical immobility without any activity • Interacting: Directing investigatory behavior toward another steer (e.g., sniffs, massage), allogrooming, or engaging in agonistic interaction (e.g., pushing, head-knocking, riding) • Drinking: Drinking water from the water trough • Feeding: Head positioned inside of the feeder with oral movement • Eliminating: Urinating or defecating • Ruminating: Chewing while not directly consuming feed
Postures
<ul style="list-style-type: none"> • Standing: Standing on all four legs • Lying: Lying down, either on belly or on one side.

internal standard of 250 μM norleucine was used in each sample and retention times were compared to physiological amino acid standard containing a mix of 41 basic, acidic, and neutral amino acids (Sigma-Aldrich) and glutamine.

Plasma was analyzed by enzyme-linked immunosorbent assay for adrenocorticotrophic hormone (ALPCO ACTH kit 21-ACTHU-E01), adrenaline (DIAsource adrenaline kit KAPL10-0100) and serotonin (DIAsource serotonin Fast Track kit KAPL10-0900). Serum was analyzed by enzyme-linked immunosorbent assay for cortisol (DIAsource cortisol kit KAPDB270). Glucose was measured using the glucose oxidase-peroxide reaction (Cayman Chemical kit 10009582). All kits were used according to instructions provided by the manufacturer.

Statistical analysis

The results were analyzed as repeated measures using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC). Covariance structures were chosen considering both the shape and range of the curve between correlations and lag time (as compared to an unstructured covariance structure), and both the Bayesian and Akaike information criterion, respectively, as described by Littell et al. (2000, 2002). Factors in the model were experimental period, animal, diet, day, and diet by day interaction. The repeated measurements were observations and variables measured on the same animal across days. When ANOVA indicated a significant ($P < 0.05$) effect for diet, differences between the control (0 mg of L-Trp/kg BW/d) and the other treatments were determined by least square differences using a Dunnett-Hsu adjustment. When ANOVA indicated a significant ($P < 0.05$) diet by day interaction, differences against the control were estimated using a Dunnett-Hsu adjustment within day.

Results

We observed no effect of diet or diet by day interaction in the percentage of observation in which steers were standing and lying. Overall, steers spent almost half the day standing and half day lying (Table 2). Results indicated no effect of diet or diet by day interaction for most of the evaluated activities (Table 3). Diet was significant in explaining differences only in time spent inactive ($P = 0.0250$). Overall, steers receiving 150 mg Trp/kg BW/d spent more time inactive than steers with the control diet ($P = 0.0305$). The animals were most of the time inactive (between 60% and 70% of the observations) and the most common activities were feeding (about 20% of the observations) and ruminating (between 10% and 15% of the observations).

There was no effect of diet or diet by day interaction for most of the evaluated blood variables (Figure and Table 4). The only significant differences detected were in the ratio of Trp:LNAA, which were lower in steers receiving 100 and 150 mg of L-Trp/kg BW/

Table 2

Standing and lying observations in steers fed increasing amounts of purified tryptophan (N = 4 steers)

Treatment ^a	Standing (%) ^b	Lying (%)
0 mg Trp/kg BW	47.03 \pm 1.231	52.97 \pm 1.231
50 mg Trp/kg BW	44.26 \pm 1.231	55.74 \pm 1.231
100 mg Trp/kg BW	48.53 \pm 1.280	51.47 \pm 1.280
150 mg Trp/kg BW	46.55 \pm 1.280	53.55 \pm 1.280

Results expressed as mean \pm standard error.

Covariance structures chosen were autoregressive for both standing and lying.

^a Treatment expressed as mg of tryptophan (Trp) per kilogram of body weight (BW).

^b Activities are expressed as percentage of observations.

Table 3

Distribution of several activities in steers fed increasing amounts of purified tryptophan

Activity ^a	Treatment (mg Trp/kg BW) ^b			
	0	50	100	150
Drinking (%)	1.35 \pm 0.370	1.96 \pm 0.370	1.54 \pm 0.377	1.17 \pm 0.377
Feeding (%)	21.7 \pm 1.70	22.2 \pm 1.70	20.4 \pm 1.73	18.8 \pm 1.73
Eliminating (%)	0.391 \pm 0.180	0.260 \pm 0.180	0.402 \pm 0.185	0.367 \pm 0.185
Inactive (%)	60.9 \pm 1.92	59.6 \pm 1.92	64.34 \pm 2.01	69.51 \pm 2.01 ^c
Interacting (%)	0.478 \pm 0.143	0.783 \pm 0.143	0.714 \pm 0.151	0.274 \pm 0.151
Ruminating (%)	15.2 \pm 1.52	15.2 \pm 1.52	12.6 \pm 1.55	9.8 \pm 1.55

Results expressed as mean \pm standard error.

Covariance structures chosen were autoregressive for drinking, heterogeneous autoregressive for feeding, heterogeneous compound symmetry for eliminating, autoregressive for inactive, autoregressive for interacting and heterogeneous autoregressive for ruminating.

^a Activities are expressed as percentage of observations.

^b Treatment expressed as mg of tryptophan (Trp) per kilogram of body weight (BW).

^c $P < 0.05$ as compared to control (0 mg Trp/kg body weight) using a Dunnett-Hsu adjustment.

d (Figure). The average Trp and glucose concentration across treatments at baseline (d0) were $3.68 \pm 0.17 \mu\text{g Trp/mL}$ and $85.6 \pm 10 \text{ mg glucose/dL}$. Serotonin, cortisol, and adrenaline concentrations across treatments at baseline were $1,667 \pm 555 \text{ ng serotonin/mL}$, $10.7 \pm 3.4 \mu\text{g cortisol/dL}$, and $15.0 \pm 4 \text{ pg adrenaline/mL}$.

Discussion

The observation of postures indicated that steers on this experiment spent almost half the day standing and half day lying (Table 2). This is different to results reported in a study on beef calves (Nakanishi et al., 1998), which observed 34.8% of lying time and 65.2% of standing time. The difference is probably due to time postweaning or rearing conditions. Calves in Nakanishi et al. (1998) were weaned at the beginning of the measuring period and were followed up to 9 days thereafter, and recently weaned calves are known to spend more time walking and less time lying (Price et al., 2003). In addition, the housing density used was much higher (around 91 kg/m²) than in our study (around 24 kg/m²), which probably reduces the space available for lying comfortable and without being interrupted by other individuals.

As mentioned in the Results, there was no effect of diet or diet by day interaction for most of the evaluated blood variables (Figure). The only significant differences detected were in the ratio of Trp:LNAA, which were lower in steers receiving 100 and 150 mg of L-Trp/kg BW/d. This observation is opposed to what we were expecting (increased Trp:LNAA in steers supplemented with Trp) and we do not have a possible explanation for it.

The average plasma Trp concentration across treatments ($3.68 \pm 0.17 \mu\text{g/mL}$) was similar to previous results of $3.73 \pm 1.23 \mu\text{g/mL}$ (Bruschetta et al., 2010) and $5.72 \pm 0.60 \mu\text{g/mL}$ (Kollmann et al., 2008), both in dairy cows. The average glucose concentration was $85.6 \pm 10 \text{ mg/dL}$, and these values remained constant throughout the period. They were also similar to results reported for beef cattle (81 mg/dL; Doornenbal et al., 1988) but higher than results from dairy cows ($63 \pm 4.6 \text{ mg/dL}$; Bruschetta et al., 2010), probably because of the high demand of glucose for lactose production in lactating animals.

In contrast to Trp and glucose, there is a wide variation on the concentrations reported for stress-related hormones in the literature. The average cortisol concentration observed in this study ($10.7 \pm 3.4 \mu\text{g/dL}$) was 10 times higher than concentrations reported for unstressed animals by Grandin (1997), but only two times higher than results reported by Doornenbal et al. (1988) and Bruschetta

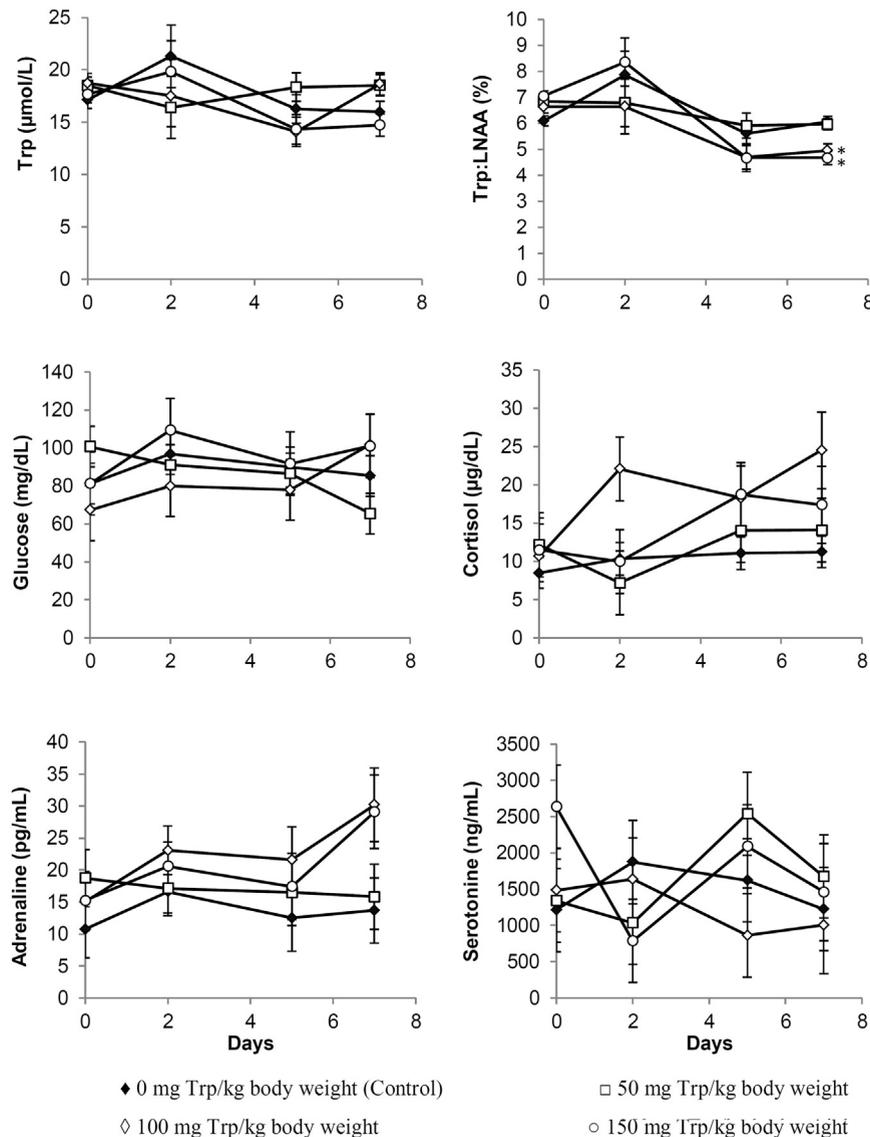


Figure. Blood variables related to stress response in steers fed increasing amounts of purified tryptophan (Trp). Error bars represent \pm SE; ratio tryptophan to large neutral amino acids (Trp:LNAAs); * $P < 0.05$ as compared to control (0 mg Trp/kg BW) using a Dunnett-Hsu adjustment. Covariance structures chosen were antedependence for Trp, heterogeneous autoregressive for Trp:LNAAs, autoregressive for glucose, autoregressive for cortisol, antedependence for adrenaline, and compound symmetry for serotonin.

et al. (2010). These authors reported cortisol concentrations around 5 $\mu\text{g/dL}$ for animals that were not subjected to stress.

Similarly, we measured $1,667 \pm 555$ ng serotonin/mL across treatments at d0, which is about 10 times and 5 times higher than reported on dairy cows by Bruschetta et al. (2010) and Kollmann et al. (2008) (170 ± 50 ng/mL and 334 ± 81 ng/mL, respectively), and more than twice the concentration measured in bulls (680 ± 33.2 ng/mL) by Gil-Cabrera et al. (2005).

There seems to be a wide fluctuation on serotonin concentration among individuals. We observed concentrations ranging from 118 to more than 5,000 ng/mL across animals and days. Other authors (Kollmann et al., 2008) reported that, although the average serotonin concentration was 334 ± 81 ng/mL, over 25% of the samples were over 1,000 ng/mL. Similarly, a wide range of serotonin concentration was observed in bulls (237 ng/mL to 1,549 ng/mL) and calves (203 to 1,969 ng/mL) by Gil-Cabrera et al. (2005).

Variation in circulating serotonin might be related to several factors. In humans, 95% of serotonin resides in the gut and serotonin released from enterochromaffin cells is virtually the only source of blood serotonin (Gershon and Tack, 2001). Several neuroactive

peptides affected serotonin release: higher circulating ghrelin reduced serotonin (Udum and Tanriverdi, 2013), whereas the release of the colonic peptide YY is at least in part responsible of increasing serotonin released from enterochromaffin cells (Kojima et al., 2012).

In addition, brain serotonergic activity is related to several behaviors such as depression, stress, aggression, and social status (Larson and Summers, 2001). Interaction between dominant and subordinate males may differentially affect serotonergic activity in different regions of the brain of the lizard *Anolis carolinensis* Voigt, most likely as a result of the stress associated with social interaction (Summers et al., 2005), and changing serotonergic activity by means of a selective serotonin reuptake inhibitor (Sertraline hydrochloride) can also revert dominant-subordinate ranking in pairs of individuals (Larson and Summers, 2001).

Thus, the wide ranges of circulating serotonin levels observed in this and other studies could be related to differences in feeding behavior and social status. If access to resources such as feed or water is restricted, both social status and differences in natural feeding behavior may be interacting to modulate the concentration

Table 4

Blood variables (expressed as mean \pm SD) related to stress response in steers fed increasing amounts of purified tryptophan (Trp)

Treatment (mg Trp/kg BW) ^a	Day			
	0	2	5	7
Trp (μmol/L)				
0	17.1 \pm 3.29	21.3 \pm 11.23	16.3 \pm 2.74	16.0 \pm 2.37
50	18.5 \pm 0.59	16.4 \pm 0.40	18.3 \pm 1.87	18.5 \pm 2.36
100	18.6 \pm 1.35	17.2 \pm 2.32	14.1 \pm 5.70	17.5 \pm 0.82
150	17.7 \pm 1.63	19.8 \pm 3.74	13.7 \pm 0.76	15.1 \pm 0.28
Trp:LNAA (%)				
0	6.09 \pm 0.643	7.86 \pm 3.234	5.61 \pm 0.671	6.06 \pm 0.438
50	6.84 \pm 0.787	6.79 \pm 1.154	5.91 \pm 0.884	5.97 \pm 0.454
100	6.70 \pm 0.292	6.66 \pm 0.353	4.70 \pm 1.527	5.09 \pm 0.208
150	7.05 \pm 0.931	8.36 \pm 1.323	4.94 \pm 0.488	4.56 \pm 0.526
Glucose (mg/dL)				
0	81.3 \pm 18.94	96.8 \pm 21.92	89.9 \pm 12.57	85.3 \pm 12.83
50	81.3 \pm 13.84	109.4 \pm 17.82	91.7 \pm 7.20	101.0 \pm 20.32
100	100.6 \pm 35.35	91.1 \pm 28.87	86.5 \pm 13.77	65.4 \pm 43.46
150	69.9 \pm 2.44	82.6 \pm 2.66	80.5 \pm 2.44	104.4 \pm 24.98
Cortisol (μg/dL)				
0	8.5 \pm 3.49	10.3 \pm 9.72	11.1 \pm 10.14	11.2 \pm 14.05
50	12.2 \pm 6.41	7.2 \pm 5.80	14.8 \pm 15.32	14.1 \pm 7.92
100	10.7 \pm 11.03	22.0 \pm 18.84	18.3 \pm 6.17	25.0 \pm 12.32
150	11.5 \pm 11.05	10.0 \pm 7.23	18.7 \pm 9.38	18.1 \pm 14.35
Adrenaline (pg/mL)				
0	10.7 \pm 4.29	16.6 \pm 12.93	12.5 \pm 11.32	13.7 \pm 9.79
50	18.7 \pm 12.34	17.1 \pm 7.35	16.5 \pm 11.62	15.8 \pm 8.27
100	15.0 \pm 13.97	23.1 \pm 6.37	21.6 \pm 29.10	34.0 \pm 33.38
150	15.2 \pm 6.28	20.6 \pm 9.15	17.4 \pm 10.04	27.3 \pm 8.13
Serotonin (ng/mL)				
0	1213 \pm 540.7	1872 \pm 1026.3	1621 \pm 851.4	1224 \pm 539.6
50	1341 \pm 853.7	1035 \pm 331.4	2542 \pm 1721.5	1671 \pm 2238.8
100	1483 \pm 1032.9	1636 \pm 1215.5	863 \pm 353.3	1012 \pm 793.0
150	2635 \pm 1668.3	786 \pm 209.5	2090 \pm 2307.0	1024 \pm 811.0

Results expressed as mean \pm standard deviation.

^a Treatment expressed as mg of tryptophan (Trp) per kilogram of body weight (BW).

of circulating serotonin. Thus, future research would have to consider the ranking of the animals and to assess local (both intestinal and brain) serotonin metabolism to better understand possible effects of Trp and serotonin in both feeding as well as social behavior. However, assessing local serotonin metabolism is limited by the need to apply invasive techniques (colonoscopy and mucosal biopsy) and/or by the need to euthanize the animals to obtain tissue samples of the brain.

Overall, results indicate that up to 150 mg of L-Trp/kg BW/d was not effective for raising plasma Trp or raising the ratio of Trp:LNAA in supplemented steers. These observations are consistent with the lack of change in most of the behavior variables analyzed, as well as with the lack of change in serotonin, cortisol, and adrenaline.

Probably, higher doses of Trp supplementation might be needed to increase plasma Trp, similar to the ones reported by Abe et al. (1991) of 200 to 600 mg of L-Trp/kg BW. However, at such higher doses and when animals are supplemented for several days, there is an increasing risk for production of skatole (a potentially toxic compound) from Trp in the rumen due to the increasing rates of amino acid degradation that these same authors reported along 15 days of supplementation with synthetic amino acids. At the doses used in this study, it is likely that Trp was extensively degraded in the rumen by microorganisms (before it reached the small intestine), or that the amount of Trp reaching circulation was not enough to surpass the capacity of the liver to degrade high circulating Trp by the rapidly inducible enzyme TDO (Badawy, 2017).

There are reports of Trp being degraded in rumen into other indole compounds (especially skatole), which could be toxic, producing acute bovine pulmonary edema and emphysema (Adeola

and Ball, 1992; Attwood et al., 2005; Deslandes et al., 2001; Schreurs et al., 2007; Yokoyama and Carlson, 1974). In our experiment, two steers had symptoms of accelerated breathing during the last days of treatment while they were resting, and a veterinarian checked for sign of respiratory disease without finding indications of fever, coughing, or nasal discharge. This happened after steers received one of the highest doses of Trp: one when supplemented with 100 and the other with 150 mg Trp/kg BW/d. This last observations and the lack of changes in Trp concentration in plasma suggest that part of the supplemented Trp might have being transformed to skatole in the rumen of the steers.

Conclusions

It was not possible to raise plasma Trp in steers using up to 150 mg/kg BW/d free Trp, and therefore, it was not possible to observe consistent changes in behavior and in circulating serotonin or other hormones and metabolites that are related to the stress response. Thus, we concluded that despite its low solubility and our previous observation of slow degradation in rumen *in vitro*, higher doses of Trp might be needed to increase plasma Trp or plasma Trp:LNAA ratio, and possibly inducing meaningful behavioral changes in steers.

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Authors' contributions: The experiments were designed by R.L. The experiments were performed by G.M. and R.L. The data were analyzed by R.L. The article was written by G.M. and R.L.

Conflict of interest

The authors declare no conflict of interest.

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