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Relationships between early experience to dietary diversity, acceptance of novel flavors, and open field behavior in sheep

Juan J. Villalba [a,*], Francisco Catanese [b], Frederick D. Provenza [a], Roberto A. Distel [b]

[a] Department of Wildland Resources, Utah State University, Logan, UT 84322-5230, USA
[b] Departamento de Agronomía and CERZOS/CONICET, Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina

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ABSTRACT

This study determined whether early experiences by sheep to monotonous or diverse diets influence: (1) plasmatic profiles of cortisol, a hormone involved in stress responses by mammals, before and after an ACTH challenge, (2) the readiness to eat new foods in a new environment, (3) general fearfulness and response to separation – as measured by the open field test (OFT) and stress induced hyperthermia (SIH) – and (4) the link between (2) and (3). Thirty, 2-mo-old lambs were randomly assigned to 3 treatments (10 lambs/treatment). Lambs in one treatment (Diversity — DV) received in successive periods of exposure all possible 4-way choice combinations of 2 foods high in energy and 2 foods high in protein from an array of 6 foods: 3 high in energy (beet pulp, oat grain, and a mix of grape pomace:milo [40:60]) and 3 high in protein (soybean meal, alfalfa, corn gluten meal). Lambs in another treatment (DV+T) received the same exposure described for DV but two phytochemicals, oxalic acid (1.5%) and quebracho tannins (10%) were randomly added within any period of exposure to foods high in energy or to foods high in protein. Lambs in the third treatment (Monotony — MO) received a monotonous balanced ration containing all 6 foods fed to the other groups. After exposure, lambs were offered a choice of the aforementioned 6 foods (DV; DV+T) or the monotonous diet (MO). Lambs were intravenously injected with ACTH 1 h after food presentation, and sampled at 1, 2, and 3 h post feeding for determinations of plasma cortisol concentrations. Reluctance to eat novel flavored foods (onion-, coconut- and cinnamon-flavored wheat bran), open field behavior, and SIH was assessed in all treatments. Lambs in MO showed greater concentrations of plasma cortisol 1 h after food presentation than lambs in the DV or DV+T treatments ($P=0.04$). However, the difference was small and no differences among treatments were detected after an ACTH challenge ($P>0.1$). Lambs in DV consumed more onion-flavored wheat bran than lambs in MO ($P=0.05$). Lambs in DV also showed a greater cumulative consumption of novel flavors on d 2 than lambs in MO (treatment × day; $P=0.01$). Lambs in DV showed lower increase in rectal temperature ($P=0.07$) than lambs in MO. Only lambs in DV exhibited a positive relationship between consumption of cinnamon-flavored wheat bran and attempts of escape ($R^2=0.58$; $P=0.02$). Our results suggest that exposure to diverse foods early in life may be less stressful than exposure to monotonous rations, as measured by plasma cortisol concentrations after food ingestion, and by changes in rectal temperature after exposure to the OFT. Lambs exposed to diverse diets early in life may also increase the initial acceptance of new flavors in novel environments relative to lambs exposed early in life to monotonous diets.

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1. Introduction

Ruminants evolved in diverse feeding environments ingesting arrays of foods of contrasting nutritional and toxicological characteristics [1,2]. Nevertheless, current intensive feeding systems are characterized by feeding animals monotonous rations and pastures. Single foods generate orosensorial and postingestive signals which cause animals to satiate [3,4], and satiety may be aversive [3,5]. The satiety hypothesis attributes changes in palatability to transient food aversions due to flavors, nutrients, and toxins interacting along concentration gradients [3]. Gustatory, olfactory, and visual neurons stop responding to the taste, odor, and sight of a particular food eaten to satiety, yet they continue to respond to other foods. If alternatives are not available, animals stop responding and intake will decrease [6,7]. Moreover, if monotony is aversive, then animal welfare may be compromised, even if monotony implies consuming a balanced diet [8].

Besides their satiating effects, monotonous diets are predictable and offer herbivores arrays of chemicals which do not change in time or space, allowing for a prompt familiarization to the sensorial and postingestive properties of those foods. In contrast, diverse foods may be challenging to herbivores as they inherently involve elements of change and

* Corresponding author at: Utah State University, 5230 Old Main Hill, Logan, UT 84322, USA. Tel.: +1 435 797 2539; fax: +1 435 797 3796.
E-mail address: juan.villalba@usu.edu (J.J. Villalba).

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unpredictability. Variability not only in the nutritional composition of food but also in the presence of phytochemicals may add a new dimension of unpredictability as by ingesting toxins animals incur in the risk of intoxication [1]. Thus, besides selecting a diet that meet nutritional needs, the presence of phytochemicals may challenge animals to build a diet which minimizes the negative impacts of toxins on the internal environment.

Fearfulness has been defined as a basic psychological characteristic that predisposes an individual to perceive and react in a similar manner to a wide range of potentially negative events [9]. Thus, fear has been related to the specific behaviors of escape and avoidance [10]. Herbivores typically prefer the familiar to the novel, and they generally regard anything novel with caution i.e., they are reluctant to eat novel foods and explore novel environments [11]. These responses may be, at least in part, a function of the different degrees of fear responses herbivores manifest toward novelty in general. In support of this, previous research suggests that there is a link between general fearfulness and response to separation, as measured by the open field test (OFT) and stress induced hyperthermia (SIH), and the readiness of sheep to eat new foods [12]. Lambs less responsive to social isolation in OFT (lower number of bleats) were less cautious at accepting novel foods than individuals more responsive to social isolation [11]. Open field tests generally consist of exposing a single animal to an empty arena surrounded by walls and recording its behavior over a certain period of time (10–15 min). This procedure may induce acute stress, reflected in hyperthermia [12] and activation of the hypothalamic–pituitary axis with the result of an increased secretion of cortisol from the adrenal gland [13]. On the other hand, chronic stress promotes a habituation of the axis resulting in attenuated cortisol responses [13].

Fear responses to new foods and locations may be influenced by an animal's previous experiences with foods. For instance, experiences during development induce life-long neurological, morphological, and (or) physiological changes in herbivores [14,15]. Providing oro-sensorial and postingestive experiences early in life may have more pronounced effects than at later stages of life [16,17]. Animals that manifest less fear toward the unfamiliar may accept more readily new foods, leading to a more diverse diet. It is likely that early experiences to a diverse array of foods may attenuate fear responses toward novelty in general, which will be reflected in a higher acceptance of new foods and environments. Indeed, the neural pathways involved in diet selection are also involved in responses to fear [18]. Animals accept new foods less readily when kept in unfamiliar environments likely to elicit fear [19]. Work with farm animals has also shown that environmental enrichment that allows animals to show a more flexible foraging behavior decreases chronic stress [20]. Thus, the objective of this study was to determine whether early experiences to food diversity or monotony influences 1) stress responses in sheep, as measured by plasmatic concentrations of cortisol before and after an ACTH challenge, 2) acceptance of novel foods in a new location, 3) general fearfulness and response to separation, as measured by the open field test (OFT) and stress induced hyperthermia (SIH), and 4) the link between open field behavior and SIH and readiness of sheep to eat new foods.

2. Materials and methods

The study was conducted at the Green Canyon Ecology Center, located at Utah State University in Logan according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval # 1464).

2.1. Animals and dietary treatments

During the study, 30 commercial Finn–Columbia–Polypay–Suffolk crossbred lambs of both sexes (2 mo of age) with an average initial BW of 29.13 ± 4.03 kg (mean ± s.d.) were penned outdoors, under a protective roof in individual, adjacent pens measuring 2.4 × 3.6 m. Throughout the study, lambs had free access to fresh water and trace mineral salt blocks. Lambs were familiar with alfalfa and barley grain, since these foods comprised the basal diet of their mothers. Animals were weaned and introduced into their individual pens and were fed alfalfa pellets for ad libitum intake and 300 g/d of barley grain for 15 d until the beginning of exposure to diverse or monotonous diets.

Early experiences to dietary treatments by all animals are described in Ref. [21]. Briefly, lambs were randomly divided into 3 treatments (10 lambs/treatment), and conditioned with different sensorial and postingestive experiences. Lambs in one treatment (Diversity — DV) were fed simultaneously an array of 4 foods from a group of 6 (3 foods high in protein: soybean meal, alfalfa, corn gluten meal; 3 foods high in energy: beet pulp, oat grain, and a mix of grape pomace:milo [40:60] (Table 1)). Lambs from this treatment received all possible 4-way choice combinations of 2 foods high in energy and 2 foods high in protein (a total of 9 combinations). Each choice combination was fed for a period of 5 consecutive days, and all periods occurred in a continuous sequence until all combinations were exhausted. Exposure to choice combinations was counterbalanced across animals such that any combination of 4 foods for each animal in a period was distributed randomly across time. Lambs in a second treatment (Diversity+Toxin — DV+T) received the same exposure described for DV but two phytochemicals, oxalic acid (1.5%) and quebracho tannins (10%) were randomly added within any period to foods high in energy or to foods high in protein (Table 1). The concentrations of phytochemicals in foods have been used safely in previous studies and represent concentrations sheep typically encounter while grazing in rangelands [22]. Finally, lambs in the treatment MO (Monotony) received a monotonous balanced ration according to NRC [23] containing all 6 foods used in DV throughout exposure (38% milo, 12% oat grain, 10% soybean meal, 22% alfalfa, 10% grape pomace, 5% beet pulp, 3% corn gluten meal). All lambs had ad libitum access to their respective treatment diets from 0830 to 1500 for 60 d.

2.2. Feeding diverse and monotonous foods and blood sampling

After exposure to the dietary treatments (d 61), 7 randomly-chosen animals within each treatment were fitted with indwelling catheters in the left jugular vein (1.4 mm i.d., 1.7 mm o.d.; Abbott Laboratories, Abbott Park, IL). On d 62 at 0800, lambs in the DV and DV+T treatments had a simultaneous offer of all 6 foods used during exposure to the dietary treatments (Table 1), whereas lambs in MO had the monotonous ration. Food refusals were collected at 1600 and intake was determined. Ten milliliters of blood was drawn into heparinized tubes from catheters 1, 2, and 3 h after food presentation. Lambs were intravenously injected with 2 IU of porcine ACTH/kg of

Table 1
Metabolizable energy (ME), Crude protein (CP), and Neutral detergent fiber (NDF) of feeds (dry matter basis) offered to lambs in three treatments (Diversity — DV; Diversity+Toxin — DV+T, and Monotony — MO).

Feeds	Nutrient composition		
	ME, Mcal/kg[a]	CP, %	NDF, %
High in energy			
Grape pomace:milo (40:60)	2.05	11.3	34.7
Oat grain	2.52	11.7	27.2
Beet pulp	2.58	10.7	41.9
High in protein			
Soybean meal	2.90	47.3	12.4
Corn gluten meal	3.12	53.7	7.10
Alfalfa	2.11	17.8	45.6
Mix offered to Monotony treatment[b]	2.44	16.0	30.6

[a] Estimated values from NRC (1985).
[b] The mix was composed by 38% milo, 12% oat grain, 10% soybean meal, 22% alfalfa, 10% grape pomace, 5% beet pulp, and 3% corn gluten meal.

$BW^{0.75}$ (Sigma-Aldrich) immediately after drawing blood 1 h after food presentation. Samples were immediately centrifuged at 3000×g for 15 min to harvest plasma and subsequently stored at −30 °C until analyses. Plasma cortisol concentration was determined using a solid-phase radioimmunoassay technique (intra- and interassay CV of 3 and 8%, respectively; Siemens, Los Angeles, CA).

On d 65, animals from all treatments were offered the same foods for 68 d (MO ration-15 d; wheat bran-15 d; corn distillers' dried grain and fescue hay-5 d; Calf Manna and rice-5 d; green peas and rolled oats-5 d; alfalfa pellets-23 d) as described by Catanese et al. [21]. Thus, differences in feeding responses and to open field tests (OFT) could be attributed to the treatments animals experienced early in life and not to the short-term effects of foods and flavors consumed prior to testing.

2.3. Intake of novel flavors at a novel location

On d 134, all lambs (59.14 ± 5.76 kg, mean ± s.d.) were moved 250 m to an adjacent research facility, unfamiliar to all lambs, where they were randomly penned in individual adjacent pens (2.4 × 3.6 m). The familiar and unfamiliar location differed in the following manner: (1) the familiar location had pens distributed in two parallel rows with a distance of 1.5 m between rows, whereas the new location had pens distributed along a the perimeter of a rectangular area (35 × 21 m), and (2) the familiar location had a protective roof, whereas the unfamiliar location did not.

Immediately after moving the lambs to the new location, they were offered 1000 g of the familiar food wheat bran mixed (2%) with onion powder (Pacific Seasonings, Inc., Kent, WA), a novel flavor, from 1300 to 1320. We wanted to assess the lambs' first response to a novel flavor in a new location so we determined how much novel flavored food was consumed the first day the novel flavor was presented for a period of 20 min. At 1320 refusals were collected and weighed and intake of the novel flavored food was determined. After collecting refusals, lambs were fed 2 kg of alfalfa pellets and no other food was offered until the next day, when onion-flavored wheat bran was presented at 1300 for a second day of testing. On the following day, lambs were fed another novel flavor mixed in wheat bran (2% coconut flavor, Lucta, S.A., Montornés del Vallés, Spain) instead of onion for 2 d, and then another novel flavor in wheat bran (2% cinnamon, Pacific Seasonings, Inc., Kent, WA) instead of coconut, for another 2 d. Procedures for offering these two novel flavors were as described before.

2.4. Open field test

On the day after the last test for "Intake of Novel Flavors at a Novel Location" ended, lambs were tested in an OFT to measure fear in a novel environment and response to separation. Tests were performed in an arena with a concrete floor under cover of a shed. The rectangular arena measured 4.6 × 2.8 m and its floor surface was marked with a grid with squares of 53 cm, which could be seen by observers outside and above the arena through an observation window, and it was screened with wooden panels 2.5 m high. Immediately before the open field test, the animal's rectal temperature (T1) was measured with a rectal probe (Traceable® Digital) lubricated with Vaseline® Petroleum jelly and held in the rectum for 1 min. The lamb was then placed for 1 min in a starting cage adjacent to the arena but separated from it by a sliding door. After 1 min, the door was opened and the lamb was placed in the arena for 10 min. Handling of test sheep prior to entry into the arena was performed quietly by the same person each time to avoid arousal before the test. During testing, two observers stood motionless outside the arena and recorded the number of 1) grid lines crossed, 2) bleats, and 3) escape attempts. After 10 min, a second measure of the rectal temperature was recorded (T2) and animals were returned to the familiar fenced area. A change in rectal temperature (CRT) was defined as T2 − T1. The floor of the arena was cleaned after each test.

2.5. Data analysis

Area under the curve (AUC) of serum cortisol concentration before and during the ACTH challenge was calculated by the trapezoidal rule [24] using the EXPAND procedure in SAS (SAS Inst., Inc. Cary, NC; Version 9.1 for Windows).

Intake of monotonous and diverse foods (as fed basis; g/kg BW), preference for each of the 6 foods in the DV and DV+T treatments ([Food intake/total food intake] × 100), plasma cortisol concentration after 1 h of feeding and AUC, food intake of each flavored food and cumulative intake of all novel flavors ingested (as fed basis; g/kg BW), and OFT variables (number of bleats, number of lines crossed, CRT, and escape attempts), were each analyzed using a split-plot design with lambs nested within treatment (DV, DV+T, and MO). Day (Intake of Novel Flavors at a Novel Location) was the repeated measures in the analysis. Day and treatment were the fixed factors and lamb was the random factor in the model.

The linear relationship between each open field behavior and reluctance to eat a novel flavored foods (amounts of novel flavored food ingested, cumulative intake of all novel flavors ingested) was assessed using an analysis of covariance with treatment (DV, DV+T, MO) as a categorical-scale explanatory factor and open field behavior and flavor intake as continuous-scale explanatory factors. The model also included the interaction between treatment and open field behavior in a repeated measures analysis. Because both intake and field behavior values were computed on a standardized scale ([Individual value − Mean]/Standard Deviation) within each treatment prior to fitting the model, the slope estimated by the model for each treatment was equivalent to the correlation coefficient for open field behavior and intake, and the interaction term (treatment × open field behavior) tested for equality of the correlation coefficients between treatments.

Analyses were computed using a mixed-effects model (The Mixed Procedure, SAS Inst., Inc. Cary, NC; Version 9.1 for Windows). The variance–covariance structure used were those (autoregressive order-1, compound symmetry, variance components) which yielded the lowest Bayesian information criterion. The model diagnostics included testing for a normal distribution of the error residuals and homogeneity of variance. Preference values (arcsin), number of lines crossed, and CRT (Box–Cox transformation) were transformed in order to meet assumptions of normality and homogeneity of variances. Means were analyzed using pairwise differences of least squares means. Differences between means were considered significant at $P < 0.10$.

3. Results

3.1. Intake of diverse v. monotonous foods

No differences in food intake were detected when lambs were offered diverse (DV, DV+T) and monotonous (MO) foods ($P > 0.10$; Fig. 1). Lambs offered a diversity of foods consumed and preferred soybean meal = alfalfa hay = grape pomace:milo = beet pulp > oats > gluten meal ($P < 0.05$) (Fig. 1).

3.2. Plasma cortisol and ACTH challenge

Lambs in the MO treatment showed higher concentrations of plasma cortisol than lambs in the DV or DV+T treatments (Treatment effect; $P = 0.04$) after 1 h of consuming their respective diets (Fig. 1). However, no differences among treatments were detected for serum cortisol response to ACTH. Serum cortisol AUC between 0 and 2 h after ACTH injection was 296 ± 29, 297 ± 24, and 271 ± 24 nmol/L per hour for MO, DV, and DV+T, respectively (Treatment effect; $P = 0.70$).

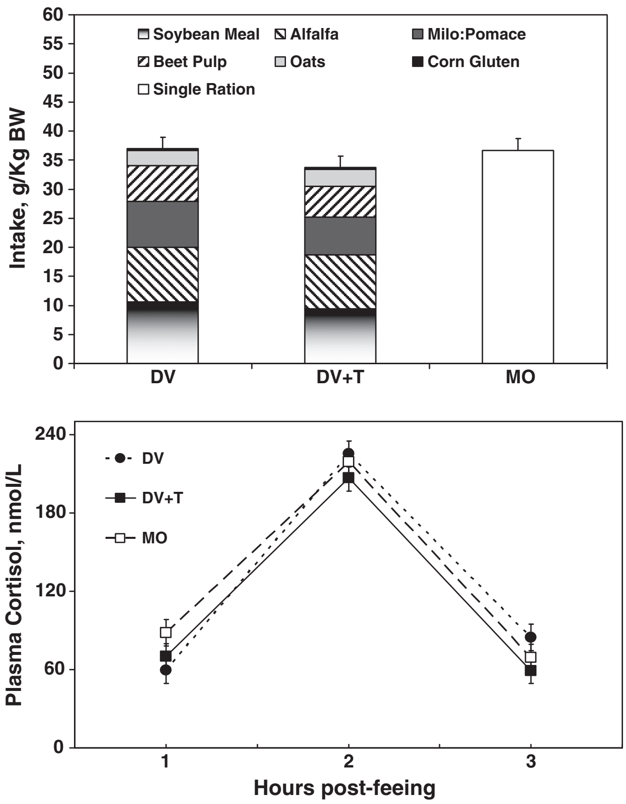

Fig. 1. Food intake (upper panel) and plasma cortisol concentration (lower panel) in three groups of lambs. Lambs in DV and DV+T were offered a choice of 6 feeds, whereas lambs in MO were fed a single ration containing all 6 feeds for 8 h. All animals were fed their respective diets and 1 h after food presentation they were intravenously injected with ACTH, and sampled at 1, 2, and 3 h after food presentation for determinations of plasma cortisol concentrations. Lambs in DV were exposed early in life to all possible 4-way choice combinations of 2 feeds high in energy (beet pulp, oat grain, and a mix of grape pomace:milo [40:60]) and 2 feeds high in protein (soybean meal, alfalfa, corn gluten meal). Lambs in DV+T received the same exposure described for DV but two phytochemicals, oxalic acid (1.5%) and quebracho tannins (10%) were randomly added within any period of exposure to feeds high in energy or to feeds high in protein. Lambs in MO received a single ration containing all 6 feeds. Intake Values are means for 10 lambs, plasma cortisol values are means for 7 lambs; SE are represented by vertical bars.

3.3. Flavor intake

Lambs in DV consumed more onion-flavored wheat bran than lambs exposed to MO (Treatment; $P=0.05$). However, no differences in flavor intake among groups of lambs were detected for coconut- and cinnamon-flavored wheat bran (Treatment; $P>0.10$) and all lambs increased intake during the second day of testing ($P<0.001$) (Fig. 2). Lambs in DV showed by d 2 a greater increase in the consumption of cinnamon-flavored wheat bran than lambs in MO (treatment × day; $P=0.001$). Lambs in DV also showed a greater cumulative consumption of flavors on d 2 than lambs in MO (treatment × day; $P=0.01$; Fig. 2). No differences in intake of single flavored foods, or cumulative intake of flavored foods was observed between DV and DV+T ($P>0.10$; Fig. 2).

3.4. Open field behavior

Lambs in DV showed a lower increase in rectal temperature than lambs in MO ($P=0.07$; Fig. 3).

3.5. Relationships flavor intake — open field behavior

Relationships among OFT variables and intake of novel flavors were not different from 0 ($P>0.10$), except for the relationship between consumption of cinnamon-flavored wheat bran and attempts of escape (attempts of escape × treatment; $P=0.07$). Lambs in DV exhibited a positive relationship ($R^2=0.58$; $P=0.02$) between these variables, whereas the other treatments showed relationships not different from 0 (DV+T: $R^2=0.10$; $P=0.34$; MO: $R^2=0.00$; $P=0.89$) (Fig. 3).

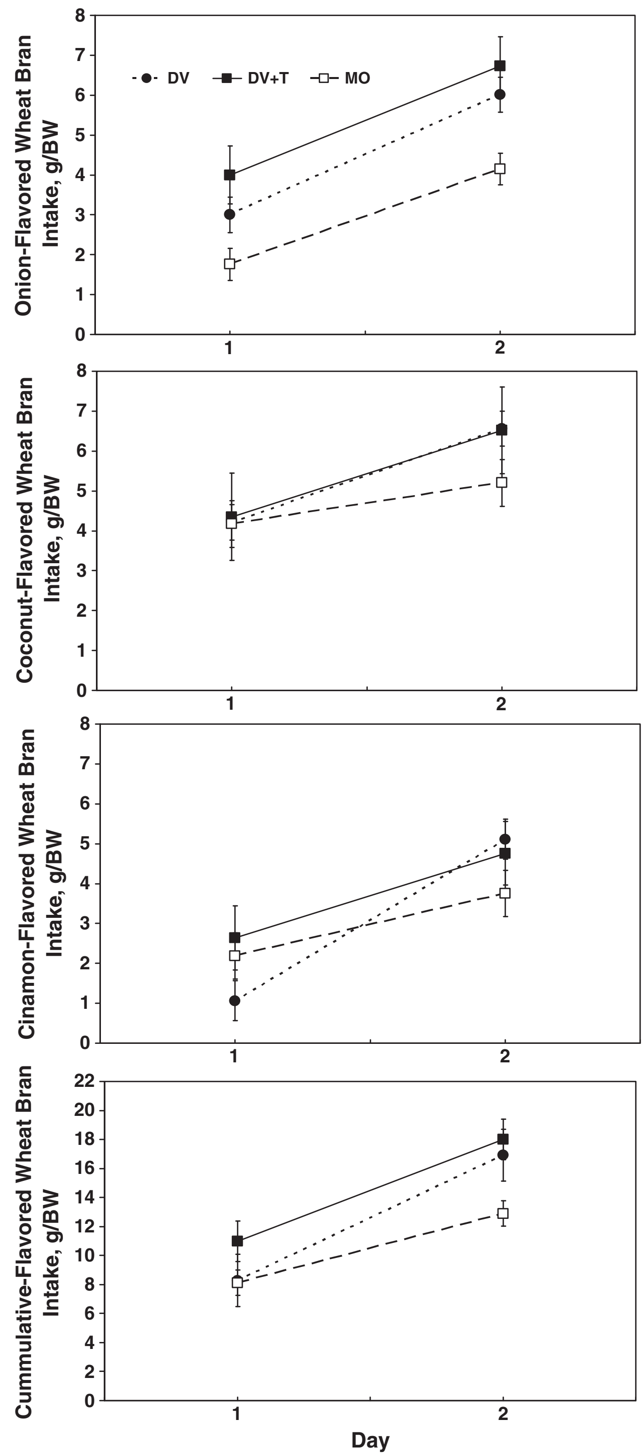

Fig. 2. Consumption of flavored-wheat bran by three groups of lambs in a novel location. Flavors were novel to the lambs. Lambs in DV were exposed early in life to all possible 4-way choice combinations of 2 feeds high in energy (beet pulp, oat grain, and a mix of grape pomace:milo [40:60]) and 2 feeds high in protein (soybean meal, alfalfa, corn gluten meal). Lambs in DV+T received the same exposure described for DV but two phytochemicals, oxalic acid (1.5%) and quebracho tannins (10%) were randomly added within any period of exposure to feeds high in energy or to feeds high in protein. Lambs in MO received a single ration containing all 6 feeds. Values are means for 10 lambs; SE are represented by vertical bars.

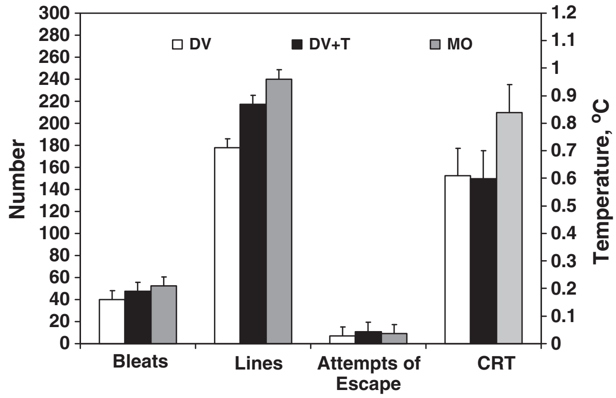

Fig. 3. Number of bleats, number of lines crossed, attempts of escape and changes in rectal temperature (CRT) in three groups of lambs when tested in an open field. Lambs in DV were exposed early in life to all possible 4-way choice combinations of 2 feeds high in energy (beet pulp, oat grain, and a mix of grape pomace:milo [40:60]) and 2 feeds high in protein (soybean meal, alfalfa, corn gluten meal). Lambs in DV+T received the same exposure described for DV but two phytochemicals, oxalic acid (1.5%) and quebracho tannins (10%) were randomly added within any period of exposure to feeds high in energy or to feeds high in protein. Lambs in MO received a single ration containing all 6 feeds. Values are means for 10 animals/group; SE are represented by vertical bars.

Treatments also differed in the relationship between changes in rectal temperature (CRT) and intake of cinnamon-flavored wheat bran during the second day of testing (CRT × treatment; $P = 0.08$). The treatment DV showed positive relationships between these variables ($R^2 = 0.71$; $P = 0.01$), whereas the other treatments showed relationships not different from 0 ($P > 0.10$; Fig. 4).

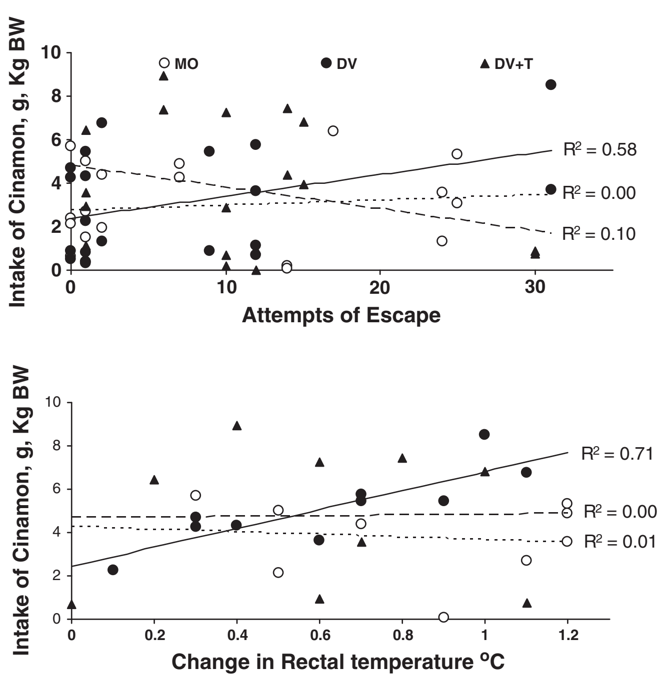

Fig. 4. Relationships between attempts of escape and change in rectal temperature, variables obtained in an open field test, and consumption of cinnamon-flavored wheat bran, a novel flavor. Lambs in DV were exposed early in life to all possible 4-way choice combinations of 2 feeds high in energy (beet pulp, oat grain, and a mix of grape pomace:milo [40:60]) and 2 feeds high in protein (soybean meal, alfalfa, corn gluten meal). Lambs in DV+T received the same exposure described for DV but two phytochemicals, oxalic acid (1.5%) and quebracho tannins (10%) were randomly added within any period of exposure to feeds high in energy or to feeds high in protein. Lambs in MO received a single ration containing all 6 feeds.

Averaged across treatments, there were positive relationships between attempts of escape and cumulative intake of flavors during the first day of testing ($R^2 = 0.17$; $P = 0.05$) (Fig. 5). Likewise, there was a positive relationship between number of lines crossed and cumulative intake of all flavors ($R^2 = 0.05$; $P = 0.07$) (Fig. 5).

4. Discussion

4.1. Stress responses to monotony

Results from the present study show for the first time in ruminants that animals exposed to a monotonous diet display greater concentrations of cortisol than lambs exposed to a diverse diet. Rats exposed to attractive and diverse, but inaccessible food cues manifest higher levels of stress than rats deprived from such exposure as measured by concentration of corticosterone [25]. An increase in plasma cortisol concentration in response to the activation of the hypothalamic–pituitary adrenal axis is one of the most typical neuroendocrine responses to stress [26]. An exogenous administration of ACTH stimulates adrenal secretion of cortisol, which may be amplified by the existence of a concurrent stressful event [27]. However, no differences among treatments were detected for cortisol concentrations (AUC) after this challenge.

The satiety hypothesis attributes changes in palatability to transient food aversions due to flavors, nutrients, and toxins interacting along concentration gradients [3,4]. Single flavors, nutrients and phytochemicals all cause animals to satiate and satiety may be aversive which limits food intake [3,5]. Thus, repeated exposure to the same

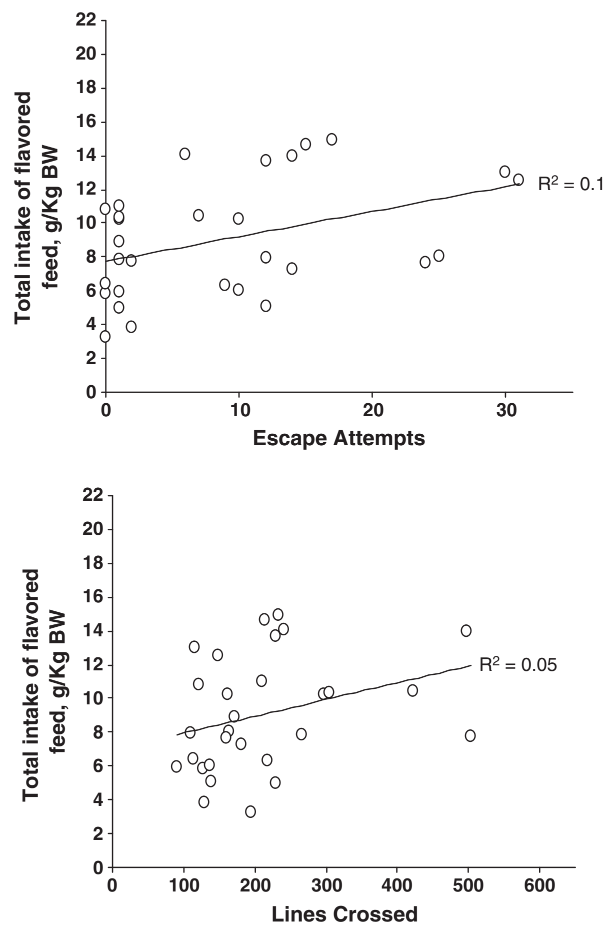

Fig. 5. Relationships between variables obtained in an open field test, and cumulative intake of novel flavors by three groups of lambs. Upper panel. Relationship between attempts of escape and cumulative intake of flavors during the first day of testing. Lower panel. Relationship between number of lines crossed and cumulative intake of all flavors.

food may lead to satiation and aversive states which may be stressful [8]. Frequent exposure to the same food may lead to sensory-specific satiety [7] which plays a key role in the regulation of food intake [28]. In contrast, diverse foods and flavors may restore the motivation to eat [29] by enhancing food acceptability [30–32].

Herbivores can balance the supply of energy and nitrogen to the rumen when offered appropriate food choices [33,34]. Thus, it is likely that food choice promotes more efficient use of the foods available and reduces the detrimental effects resulting from excesses and imbalances of nutrients in single foods [35]. The opportunity offered by multiple foods at balancing nutrient intake as a function of an animal's particular requirements and susceptibilities may be another reason stress declines with food diversity relative to food monotony [8,36].

Measurements of plasma cortisol in blood samples obtained before, during and after stress are useful in assessing stress in lambs [37]. However, responses to stressful situations such as castration, isolation, and restraint seem to yield greater concentrations of cortisol in lambs (140–270 nmol/L range [37]) than responses to food monotony observed in the present study (88 nmol/L after 1 h of ingesting a monotonous ration). This suggests that the stress level achieved during exposure to a monotonous ration is of lower magnitude than that experienced during other stressful situations.

4.2. Avoidance of novel flavors in a new environment

Besides experiencing satiety to monotonous diets, herbivores experience fear to novel foods and events which may also affect feeding behavior and the acceptance of new foods. Even within uniform groups of animals, individuals manifest different degrees of avoidance to novel foods [36]. These responses may be, at least in part, a function of the different degrees of fear responses herbivores manifest toward novelty in general. For instance, animals accept new foods less readily when kept in unfamiliar environments likely to elicit fear [19]. Our results suggest these feeding responses in novel environments are also influenced by the dietary experiences animals had earlier in life.

Experiences early in life to food diversity or monotony influence how herbivores accept novel foods later in life. For instance, lambs exposed early in life to the same ration but presented in monotonous or multiple flavors modify their initial acceptability and preference for novel foods [38]. Likewise, lambs exposed to a diverse array of foods early in life accept novel foods and flavors more readily than lambs exposed early in life to monotonous rations [21]. The initial neophobic response to novel foods did not differ between groups of lambs with contrasting experiences to food diversity. However, animals exposed early in life to diverse foods consumed greater amounts of novel flavors and foods in ensuing days than animals exposed early in life to food monotony [21]. Consistent with this observation, lambs in DV showed greater intakes of onion-flavored wheat bran and greater cumulative intake of flavored foods during d 2 of testing, suggesting acceptability of new flavors in a new environment occurred at a greater rate for DV than for MO. Thus, experiences early in life to food diversity may attenuate rejection of novel flavors even during the first time of exposure in a novel location. Differences in responses to coconut- and cinnamon-flavored wheat bran were not significant among treatments, although animals in DV increased intake of cinnamon from d 1 to d 2 to a greater extent than lambs in MO, again reflecting the greater acceptance of novel flavors across time.

Results from this study are conservative in the sense that after exposure to the dietary treatments all lambs were offered different foods for 68 d before testing for intake of novel flavors and open field behavior. This procedure may have attenuated differences among treatments, as lambs in MO also experienced novel foods during this period. The procedure (all lambs offered the monotonous feed for 15 d and alfalfa pellets for the last 23 d of the period) was performed to ensure that differences in feeding responses and behaviors in OFT could be attributed to the treatments animals experienced early in life and not to the contrasting short-term effects of different foods and flavors consumed prior to testing. Exposure to other foods during the period (e.g., wheat bran, corn distillers' dried grain, fescue hay, Calf Manna, rice, green peas and rolled oats) was carried out to test for the short-term effects of the treatments on acceptance of novel foods at the same location where exposure to the dietary treatments took place [21].

4.3. Open field test

The OFT was originally developed for rodents [39], and the behavior in the test is mainly determined by the conflict between exploration and aversion to a new environment [40]. It is generally accepted that individual differences in rodent behavior in the OFT are caused by differences in general fearfulness; animals showing less exploration and a higher frequency of defecation and urination are thought to be more fearful [41,42]. In social animals such as sheep behavior in OFT results from both exposure to novelty and separation from peers [43]. Individual differences in sheep behavior when animals are isolated from conspecifics are at least partially explained by individual differences in fearfulness, with animals showing more exploration and a higher frequency of escape attempts when isolated from flockmates [44]. No differences in attempts of escape or exploration between groups of lambs were detected in the present study.

It has been suggested that results of OFT in farm animals should be validated using physiological parameters such as stress-induced hyperthermia (SIH), as independent measures of fear [45]. Stress-induced hyperthermia has been described in a variety of mammals, including ungulates and it is considered to be a simple and species-independent phenomenon associated with encountering stressful stimuli [44,46]. Interestingly, lambs in MO showed a higher increase in rectal temperature after being exposed to the OFT than lambs in DV. If the increase in rectal temperature is a result of SIH our results suggest that lambs exposed early in life to a monotonous diet are more fearful than lambs exposed to a diverse diet. An alternative explanation to SIH is an increase in rectal temperature associated with physical exercise. In our experiment, lambs in MO also displayed the highest number of lines crossed, which is consistent with this hypothesis. However, SIH has been attributed to an increase in body temperature due to a psychological stress not caused by physical activity [47]. In addition, even those animals that showed the highest ambulation scores did not display very intense physical activity. Moreover, the experiment was carried out in the fall under relatively moderate temperatures and sheep are fairly resistant to heat stress [48]. Also, no animal was seen panting during the experiment and heat stress usually induces panting in sheep [49]. Finally, our results on plasma cortisol during feeding monotonous or diverse diets are also consistent with the finding on SIH suggesting lambs in the MO treatment were more stressed during feeding and more fearful in an OFT than lambs in the DV treatment.

Our results suggest early exposure to food monotony in lambs enhanced plasma cortisol and SIH, typical indicators of acute stress [37,44,46], relative to early exposure to food diversity. However, feeding a monotonous diet for a prolonged period of time presumably induces chronic stress which may attenuate the activation of the hypothalamic–pituitary axis. This contrast may explain why differences between treatments were modest 1 h after feeding or non-significant during an ACTH challenge.

We hypothesized that the presence of phytochemicals fed to one treatment of lambs (DV + T) would add a new dimension of unpredictability thus challenging animals to a greater extent than exposure to a diverse array of safe foods (DV). Nevertheless, only lambs in DV differed in flavor intake and open field behavior from those in MO. Thus, the presence of phytochemicals in diverse foods attenuated the differences observed between treatments exposed to diverse or monotonous rations, possibly due to the prevalence of the aversive effects of toxins.

4.4. Open field test and reluctance to eat novel foods

Negative relationships between number of bleats in OFT and amount of novel food consumed by different groups of sheep have been reported [12]. In contrast, no general pattern for a relationship between acceptance of novel flavors and OFT behavior emerged in this study. In general, relationships between variables in OFT and ingestion of flavored-wheat bran by different groups of lambs were weak and non-significant. Only positive and weak relationships between consumption of cinnamon-flavored wheat bran and attempts of escape or changes in rectal temperature for lambs in DV were observed, but consumption of this particular flavor by different groups of lambs did not differ during testing. Even when lambs in different treatments showed contrasting responses in flavor acceptance, SIH, and behaviors in OFT, relationships among these variables were not evident. It is likely that there is no relation between fearfulness in OFT and acceptance of novel foods when lambs are exposed to diverse or monotonous diets early in life, or that the measurements selected were not the most appropriate to observe a relationship.

5. Conclusions

Our results suggest that exposure to diverse foods early in life may be less stressful than exposure to monotonous rations, as measured by plasma cortisol concentrations after food ingestion, and by changes in rectal temperature and ambulation scores after exposure to OFT. Our results also show that reluctance to ingest new foods in a new environment may be influenced by early exposure to diverse or monotonous foods. Thus, it may be possible to reduce stress and increase acceptability of novel foods and environments by exposing animals early in life to a diversity of foods.

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