



# Mechanisms of intake induction of a low-nutritious food in sheep (*Ovis aries*)

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## ABSTRACT

Intake induction refers to the phenomenon by which animals increase consumption of a less-valued meal when followed by a highly-preferred food relative to when followed by no food or by the same less-preferred food. In the Training phase of the present experiment, we assessed the induction effect in sheep using a within-subject design where learning could be tested while controlling for digestive state. Results showed that, once intake reached stability, subjects ate more low-nutritious food (oat hay) when followed than when preceded by a preferred food (soybean meal), supporting the learning hypothesis of induction. The objective of the second, Revaluation, phase of the experiment was to explore the associative mechanism of induction, for which we paired gastrointestinal malaise caused by lithium chloride intoxication with consumption of soybean meal or a control food (wheat bran). Despite subjects partially rejecting soybean meal relative to controls after the aversive conditioning protocol, oat hay consumption seemed unaffected by soybean meal devaluation. We conclude that intake induction in sheep may rely on changes in hedonic properties of the low-nutritious food based on its association with post-ingestive feedback from the preferred food (hedonic hypothesis), but not on an explicit anticipation of the latter (signalling hypothesis).

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## 1. Introduction

Consumption of single foods, or choices among food alternatives is not only determined by the intrinsic properties of the available food incentives (e.g., nutritional composition), but also by the interaction of foods at different levels in the experience of the animal (Flaherty, 1996; Provenza et al., 2003). The specific array of foods encountered and the sequence of encounters could turn out to be crucial in determining animals' level of consumption of each food and its nutritional consequences (e.g., see Bergvall and Balogh, 2009; Bergvall et al., 2006, 2007; Mote et al., 2008; Papachristou et al., 2007; Villalba and Provenza, 2005). These contextual effects may occur because of the digestive interaction among foodstuffs, associative learning and comparison processes, or the interplay between these mechanisms (Provenza et al., 2003).

In the present study, we focused on a phenomenon called intake induction or facilitation that results when animals repeatedly (e.g., across days) experience sessions in which consumption of a less-preferred food is intimately followed by a highly-preferred food. The intake induction effect consists of increased consumption of the

low-valued meal relative to controls that do not have access to the preferred food (e.g., Flaherty and Grigson, 1988; Weatherly et al., 2005a). For example, rats having 3-min access to a solution of 0.15% saccharin increased their licking rate of this solution when they had a subsequent 3-min access to a highly-preferred 32% sucrose solution in the same drinking spout, relative to when they just had the 0.15% saccharin solution in both periods (Weatherly et al., 2005a). Note that on similar experimental circumstances, researchers have frequently found the opposite of induction, namely negative anticipatory contrast (i.e., the partial suppression of behaviour directed towards the first food in a two-food sequence where the second food is of higher hedonic value than the first; see Flaherty, 1996, pp. 108–128). Two factors known to affect whether induction or contrast occur are the level of subjects' food deprivation (a high degree of food deprivation is associated with induction) and the location of reward delivery (if the place and conditions of delivery of the first and second reinforcers are very similar, induction is the most likely outcome; Weatherly et al., 2005a). In the present experiment with sheep, these two factors were taken into account in an attempt to cause induction (i.e., subjects had restricted access to food, and the same location and type of food bowl were used for the presentation of experimental foods; see Section 2.1 for further details).

We believe that research on intake induction in sheep may not only contribute to the understanding of the mechanisms and factors that modulate ingestive behaviour, but may also have

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**Table 1**

Schematic representation of an experimental session as a function of flavour group and treatment. All subjects experienced sessions of both treatments (i.e., within-subject design), but flavour groups were independent (between-subject factor). Sessions consisted of two parts of 20 min each. The oat hay presented in the first part of a session was removed before the second event. Pf: oat hay with parsley flavour; Of: oat hay with oregano flavour; S: 0.4% BW of soybean meal; EF: empty feeder.

	A session in treatment soybean 20 min → 20 min	A session in treatment empty feeder 20 min → 20 min
Group parsley (n = 12)	Pf → S	Of → EF
Group oregano (n = 12)	Of → S	Pf → EF

practical implications for animal production. For example, increasing livestock's dietary breadth by inducing a higher preference for lower-quality foodstuffs could be used to enhance food ingestion and reduce searching time when highly preferred forages are scarce, avoiding declines in sustainability and biodiversity of grasslands which result from overgrazing of more nutritious species. In the case of ruminants (e.g., sheep, goats), an increment on intake of low-nutritious forages may be observed when low levels of nutritional supplements are offered during foraging (Moore et al., 1999). However, to our knowledge, there has been no research exploring the extent to which learning contributes to the effect of nutrient supplementation on consumption of low-nutritious foods in ruminants. More specifically, the objectives of this study were to assess in sheep: (1) intake induction of oat hay (low-nutritious food) conditioned with the subsequent supply of either soybean meal – a highly-preferred protein supplement – or an empty feeder, controlling the subjects' current digestive state (approached in the Training phase of the experiment); and (2) the associative mechanism involved in the induction effect (approached in the Revaluation phase).

### 1.1. Goal 1: Assessing learning while controlling for digestive state

Induction effects on food consumption presumably rely on the Pavlovian contingency implicit in the feeding sequence. This means that some kind of learned association between distinct aspects of the subsequent food incentives is highly likely to underlie the expression of this phenomenon (Flaherty et al., 1994). Nonetheless, the behavioural mechanisms of induction are not fully understood yet (Flaherty and Grigson, 1988; Weatherly et al., 2000, 2002, 2003, 2004, 2007). Moreover, it is likely that digestive interactions contribute to intake induction beyond learning. This seems particularly true in ruminants such as sheep in which some foods remain in the digestive tract for hours before passage or absorption; indeed, digestive synergies or antagonisms among foods eaten in sequence can affect food acceptance and preference in these animals (Matejovsky and Sanson, 1995; Sanson et al., 1990; Villalba and Provenza, 2005).

In this first phase, we used a within-subject design in which the same subjects experienced two types of sessions. In *soybean sessions*, oat hay was presented for 20 min and then was followed by 0.4% BW (body weight) of soybean meal. Soybean meal offered at this level increases intake of fibre-rich low-nutritious food in sheep (e.g., oat hay; Matejovsky and Sanson, 1995; Moore et al., 1999). In *control sessions* (also referred to as *empty feeder sessions*), the initial 20 min of oat hay were followed by the presentation of an empty feeder. Dried parsley and oregano were used at very low concentrations (see Section 2.1 for more details) to flavour oat hay; these flavour cues were indicative of the type of session, that is, whether soybean meal or an empty feeder followed in the second part of a session (see Table 1). Animals experienced one session per day and 3 consecutive days with the same type of session, then

switching to the other type of session for another 3 days, and then switching again for a period of 24 days (we called *cycle* the set of six sessions which included three consecutive sessions of each type).

According to the learning hypothesis, at asymptotic learning, oat hay consumption should be modulated by flavour cues predictive of the next event in a session. More specifically, oat hay intake was expected to be higher in soybean sessions relative to empty feeder sessions by the end of training.

As mentioned before, a digestive process could also be involved in intake induction. It is known that ruminants can consume higher quantities of a low-nutritious food (e.g., oat hay or mature grass) when fed protein supplements (e.g., soybean meal) since the latter favourably affects rumen microorganisms responsible for fibre degradation (Matejovsky and Sanson, 1995; Sanson et al., 1990). In the present protocol, oat hay consumption could be stimulated by previous access to soybean meal. In this sense, the crucial variable to explain intake induction could be whether subjects ate soybean meal in the last 24 h before oat hay consumption (i.e., in the previous session).

To contrast the learning against the digestive hypotheses, we relied on within-subject comparisons of oat hay intake in sessions 1 and 4 of each cycle. In the first and the fourth session of a cycle, access to soybean meal (or the empty feeder) either preceded (i.e., was accessed in the previous session) or followed oat hay consumption, but not both. Therefore, the analysis of oat hay intake in the first and fourth session of a cycle allowed us to assess whether consumption was more strongly modulated by access to soybean meal in the preceding session or in the second part of the current session. In addition, it was possible that learning and digestive processes would be complementary and not exclusive. The interaction between learning and digestive supplementation predicts an upward trend in oat hay consumption across soybean sessions of the same cycle (i.e., if the effect of nutrient supplementation accumulates across days of soybean meal access), and a downward trend across control sessions of the same cycle (i.e., if more days since last soybean meal access affects current oat hay digestion). We tested these predictions by measuring the stability of oat hay consumption across subsequent sessions of the same type (be them soybean meal or control sessions), and also relative to oat hay intake in sessions of the other type.

### 1.2. Goal 2: Associative mechanisms of intake induction

In the literature of induction and contrast phenomena, authors have discussed several mechanisms that may underlie the effects of specific training sequences on consumption and preference (e.g., Flaherty et al., 1995; Lucas et al., 1990; Weatherly et al., 2005a; Williams, 1991). We here focused on whether behaviour in induction protocols might be controlled by changes in either the signalling or the hedonic value of the first food in the sequence.

The signalling value of a stimulus involves the predictive information conveyed by the stimulus that allows the animal to anticipate the quality and quantity of future reinforcing events. Authors such as Williams (1991, 1992) and Flaherty et al. (1995) have proposed that rats' increased preference for a solution used to being followed by a preferred food may indicate that choice behaviour was controlled by the signalling value of the food. According to these authors, subjects increment their preference for the target food because it signals a hedonically preferred subsequent meal. (By hedonic value, we refer to the presumed reinforcing consequences of tasting and digesting a particular substance; e.g., how pleasurable to the animal that substance results.) In other words, cues from the initial, low-quality, food (e.g., its flavour) may remind the animal of the subsequent event in the session (i.e., a stimulus-stimulus association in terms of Pavlovian conditioning). When the animal anticipates a highly preferred food, this memory

may enhance consumption of and preference for the predictive food (i.e., the low-quality incentive). The devaluation of the second food in the sequence by means of intoxication or satiety represents a crucial manipulation to evaluate this hypothesis (e.g., see Holland and Straub, 1979 for a Pavlovian conditioning example; see Colwill and Rescorla, 1985 for an instrumental conditioning example), because if the second meal is no longer preferred (as a result of the devaluation procedure), then its anticipation should adversely affect the behaviour directed towards the initial food that predicts it.

In contrast to the signalling hypothesis, we considered the hedonic hypothesis. This latter hypothesis consists of considering that the induction effect may respond to animals partially attributing the post-ingestive feedback of the second, preferred, food to the initial, low-nutritious, meal. This could be so because of the close temporal proximity between both ingestive events. Yeaesley et al. (2006) suggested that animals may incur in this type of associational errors between closely ingested foods since information pairing between food attributes and post-ingestive cues is not perfect and there is evidence that may be affected by the temporal separation between feeding events (Duncan and Young, 2002). In this sense, the initial food may not be preferred because it predicts the highly-nutritious food, but because of its association with the reinforcing post-ingestive properties of the highly-nutritious food. According to this reasoning, an independent devaluation of the highly-nutritious food should not affect consumption of and preference for the initial low-nutritious food.

The Revaluation phase was conducted to explore the signalling hypothesis as a possible explanation of intake induction in sheep. After the Training phase, the same subjects were presented with a pre-conditioning choice test, namely a choice between oregano- and parsley-flavoured oat hay. After this choice (see Section 2.1 for further details), we induced gastric sickness in all animals by administration of the toxicant lithium chloride (LiCl) after they ingested either soybean meal (for half the subjects – Treatment) or wheat bran (i.e., a familiar food consumed by the remaining animals – Control). The pairing of a food with the negative gastric effects of LiCl is known to induce a transient aversion to that food in sheep (see du Toit et al., 1991) and, therefore, to devalue its hedonic and incentive properties. When subjects recovered from the induced intoxication, we assessed preference for flavoured oat hay again (i.e., the post-conditioning choice test). We also evaluated consumption of soybean meal in an *ad libitum* 5-min test to measure the efficacy of the aversive conditioning procedure.

The rationale of these tests was as follows: if the signalling hypothesis is the appropriate explanation of the induction effect and flavour preference, then these effects should disappear, or at least diminish, after soybean meal is devaluated by the treatment with LiCl; this is so because we would expect sheep to stop preferring the flavour that reminds them of the now unattractive supplement. Otherwise, the hedonic hypothesis is the most likely explanation for intake induction in sheep.

## 2. The experiment

### 2.1. Methods

#### 2.1.1. Animals and materials

The experiment took place at the “Centro de Recursos Naturales Renovables de la Zona Semiárida” (CERZOS), located in Bahía Blanca (38°44'S; 62°16'W), Argentina, from April to May 2009. All maintenance and experimental protocols fulfilled animal welfare regulations of the Universidad Nacional del Sur, Bahía Blanca, Argentina, and adhere to the ASAB/ABS guidelines for the use of animals in research (2006).

Twenty-four male Corriedale sheep (*Ovis aries*), of  $6 \pm 0.3$  (mean  $\pm 1$  SD) months of age and  $30.0 \pm 4.5$  kg of body weight, were

brought to the CERZOS facilities in early April 2009. They spent the first 2 weeks in a communal enclosure (200 m<sup>2</sup>). One week before the start of the experiment animals were transferred to individual pens (3 m<sup>2</sup>) under a protective roof. Throughout the communal as well as the individual housing period, subjects were fed alfalfa pellets in the afternoon and had free access to fresh water and mineral salt. When in the communal enclosure, subjects were also fed ground corn, soybean meal, and pelleted wheat bran. Each food was presented during different days to familiarize animals with the foods. Thus, by simply monitoring the herd, we were able to assure that all subjects consumed all food types presented.

#### 2.1.2. Procedure

**2.1.2.1. Training phase.** Subjects were randomly assigned to a flavour group – either group parsley ( $n=12$ ) or group oregano ( $n=12$ ) – balanced by body weight. The main within-subject factor was whether oat hay was followed by 0.4% BW of soybean meal (treatment soybean or TS) or by an empty feeder (treatment empty feeder or EF) in a session. Soybean meal followed parsley flavoured oat hay for subjects in group parsley, and oregano flavoured oat hay for subjects in group oregano. An empty feeder followed oregano flavoured oat hay for subjects in group parsley, and parsley flavoured oat hay for subjects in group oregano (see Table 1 for a schematic representation of an experimental session from each treatment and flavour group). Parsley and oregano flavours were mixed with oat hay at a concentration of 2% (wt/wt), since sheep can be conditioned with flavour cues dosed at this level (Villalba and Provenza, 1996).

Subjects were presented with one training sessions per day that started at 0900. Training sessions in both treatments consisted of two parts of 20 min each, separated by 5 min (the time it took to remove the bowls of the first part and place the bowls corresponding to the second part of the session). The bowls where food was offered in each part of a session were almost identical in terms of shape and colour (half 20 L barrels of black plastic), and their location – either the right or left position in the pen – was determined randomly for each subject and session. During the first part of a training session, all subjects had *ad libitum* access to oat hay [metabolizable energy (hereafter “ME”): 7.11 MJ/kg, crude protein (hereafter “CP”): 4.8%, and neutral detergent fibre (hereafter “NDF”): 76.3%], whereas during the second part they had access to 0.4% BW of soybean meal (ME: 13.30 MJ/kg, CP: 45.8%, and NDF: 16.1%) or were presented with an empty feeder. All animals always consumed the whole amount of soybean meal offered. At 1800 all subjects had 45 min of *ad libitum* access to alfalfa pellets (ME: 9.50 MJ/kg, CP: 20.1%, and NDF: 42.6%). We measured daily consumption of oat hay fed in training sessions, and of alfalfa pellets in the afternoon meal, as the difference between offered and refused amounts. Individual daily consumption data were corrected by each animal's body weight, and expressed as grams consumed per kilogram of body weight (g/kg BW).

On any given day, half of the subjects experienced oregano flavoured oat hay (i.e., six subjects from each flavour group), and the other half experienced parsley-flavoured oat hay; half of the subjects in each flavour group received soybean meal after oat hay, while the other half experienced an empty feeder in any given session. Thus, the order of exposure to each flavour and to each treatment was counterbalanced among subjects. Each subject experienced sessions of a given treatment (TS or EF) during 3 consecutive days, and then sessions of the alternative treatment in the following 3 days. Six-session cycles were run until consumption of both parsley and oregano flavoured oat hay was stable as evidenced by a non-significant session  $\times$  treatment interaction in the ANOVA. It took a total of four cycles to reach stability (i.e., 24 sessions, 12 with oregano flavoured oat hay and 12 with parsley flavoured oat hay).

**2.1.2.2. Revaluation phase: Pre-conditioning choice test, conditioning of food aversion, and post-conditioning choice test.** The day after the fourth cycle of training finished, all subjects experienced a pre-conditioning choice test, in which they had *simultaneous* access (for 20 min) to oregano- and parsley-flavoured oat hay. The amount of each flavour offered and refused was weighed and used to estimate subjects' preference. After the pre-conditioning choice test, half the subjects in each flavour group were randomly assigned to either treatment "soybean meal + LiCl" (hereafter S + LiCl,  $n = 12$ ) or treatment "wheat bran + LiCl" (hereafter W + LiCl,  $n = 12$ ). For 4 consecutive days, at 0900 each day, subjects from S + LiCl received 0.4% BW of soybean meal and subjects from W + LiCl received 0.4% BW of wheat bran. Subjects had had previous experience with wheat bran as they were offered this food in the communal enclosure where they ate it avidly. Thus, wheat bran represented a familiar acceptable food for these subjects. In addition to the morning feeding session, all subjects were fed *ad libitum* alfalfa pellets for 45 min in the afternoon to complement their diets. On the fourth day we ran the aversive conditioning protocol as follows: once subjects had finished eating their daily ration of soybean meal or wheat bran (which took less than 5 min), they received an intra-ruminal infusion of LiCl (45 g LiCl/litre of water), administered by drench, at a dose of 5 ml of LiCl solution per kg BW (i.e., 0.225 g LiCl/kg BW). This dose of LiCl is known to induce acute gastric sickness in sheep, and when administered following the ingestion of a particular food animals develop a transient aversion to that food (Duncan and Young, 2002; du Toit et al., 1991; Villalba and Provenza, 2000). No other food was offered until the next day. All lambs were fed alfalfa pellets in the afternoon for 2 consecutive days after the treatment with LiCl.

In the morning of the third day after the intoxication event, we ran the post-conditioning choice test as described before for the pre-conditioning choice test. From 0900 to 0920 subjects had a simultaneous presentation of two food bowls, one with *ad libitum* amounts of parsley-flavoured oat hay and the other with *ad libitum* amounts of oregano-flavoured oat hay. We measured the amount of food consumed from each bowl to estimate preference. One hour after the end of the post-conditioning choice test, we gave all subjects *ad libitum* access to soybean meal for 5 min to confirm that subjects from S + LiCl had developed a conditioned aversion to that supplement relative to subjects from W + LiCl. The expectation was that subjects from S + LiCl would eat smaller amounts of soybean meal than subjects from W + LiCl.

### 2.1.3. Statistical analyses

**2.1.3.1. Training phase.** Average consumption of oat hay during each training cycle was analysed using an ANOVA with flavour group (groups parsley and oregano) as a between-subject factor, and oat hay flavour (parsley and oregano flavour) as a within-subject factor. Treatment differences and stability in oat hay consumption (g/kg BW) in the last training cycle were assessed using an ANOVA with flavour group as a between-subject factor, treatment (TS and EF) as a within-subject factor, and sessions as repeated measures. To contrast predictions from the learning and the digestive hypotheses of induction, we compared oat hay consumption in the first and the fourth session of each cycle. We ran an ANOVA of oat hay consumption in sessions one and four of each cycle with soybean as a within-subject factor (i.e., whether the supplement was accessed in the previous or the current session), flavour group as a between-subject factor, and cycle as repeated measures. Tukey HSD test was used to assess the soybean effect in each cycle.

**2.1.3.2. Revaluation phase: Pre-conditioning choice test, conditioning of food aversion, and post-conditioning choice test.** The frequency of subjects with a preference for (i.e., higher consumption of) parsley flavoured oat hay in both (i.e., pre and post-conditioning) choice tests was analysed using Fisher's exact test, with flavour

group as a factor. In addition, we used Mann–Whitney *U* tests to analyse the proportion of parsley flavour intake over the overall intake in choice tests with flavour group as the between-subject factor. Choice consumption of the flavour used to being followed by soybean meal during training was analysed with a  $2 \times 2 \times 2$  ANOVA with flavour group and aversive conditioning treatment (i.e., S + LiCl and W + LiCl) as between-subject factors, and pre/post-conditioning choice test as a within-subject factor. Soybean meal intake after the aversive conditioning session was analysed with an ANOVA, using the aversive conditioning group as a between subject factor. The  $\alpha$  value was set at the 0.05 level.

## 2.2. Results

### 2.2.1. Training phase

Fig. 1 shows mean oat hay intake of groups parsley and oregano across the training phase. Subjects found the parsley flavour more palatable than the oregano flavour at the beginning of training (i.e., in the first cycle). The ANOVA of oat hay consumption in the first cycle (averaged across sessions of the same type) with oat hay flavour and flavour group as factors, showed a significant effect of flavour ( $F_{1,22} = 15.95$ ,  $P < 0.001$ ), but neither an effect of flavour group ( $F_{1,22} = 0.54$ ,  $P = 0.47$ ) nor of flavour  $\times$  flavour group interaction ( $F_{1,22} = 0.05$ ,  $P = 0.83$ ). In contrast, this same analysis of consumption data in cycles 2, 3, and 4 showed significant flavour  $\times$  flavour group interactions (cycle 2:  $F_{1,22} = 4.35$ ,  $P < 0.05$ ; cycle 3:  $F_{1,22} = 50.51$ ,  $P < 0.001$ ; and cycle 4:  $F_{1,22} = 28.49$ ,  $P < 0.001$ ), meaning that subjects from each flavour group began responding differently to each flavour after some training.

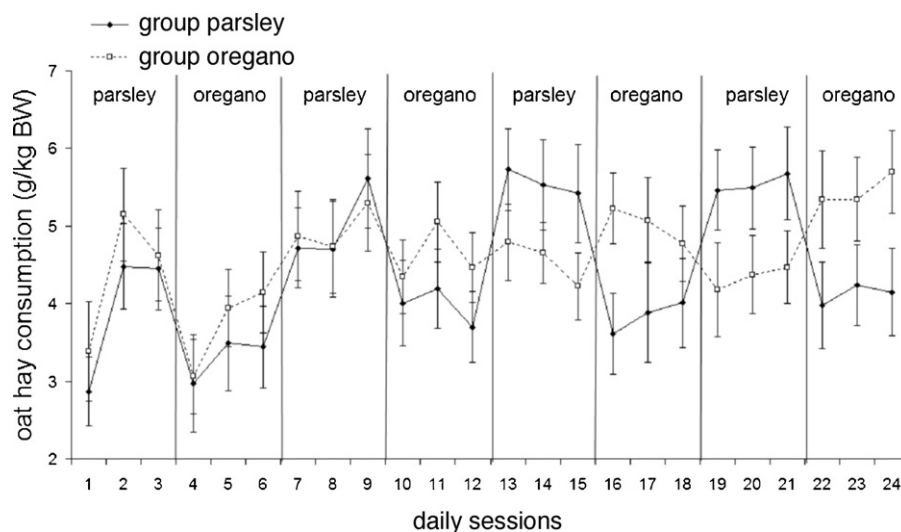
In cycles 3 and 4, group parsley presented a mean consumption of parsley flavour higher than that of oregano flavour, while the reverse was true for group oregano (see Fig. 1). Collectively, our results show that by the end of training, subjects consumed more of the flavour followed by the supplement than of the flavour followed by the empty feeder. The ANOVA of oat hay consumption in the last training cycle with treatment, flavour group, and sessions as factors showed a main effect of treatment ( $F_{1,21} = 25.32$ ,  $P < 0.001$ ) and confirmed the stability in consumption for the last three sessions of each treatment (session,  $F_{2,42} = 1.53$ ,  $P = 0.23$ ; session  $\times$  flavour group, session  $\times$  treatment, and sessions  $\times$  flavour group  $\times$  treatment interactions, all  $F_s \leq 1$ ).

To contrast predictions from learning and digestive hypotheses of induction, we compared oat hay consumption in the first and the fourth session of each cycle. Oat hay consumption increased from cycle 1 to cycle 2, but seemed independent on whether soybean meal followed oat hay or had been eaten in the previous session. By cycles 3 and 4, subjects consumed more oat hay when followed than when preceded by soybean access (see Fig. 2), thus supporting the learning hypothesis against the digestive stance. The ANOVA of oat hay consumption in sessions one and four of each cycle with soybean (whether the supplement was accessed in the previous or the current session), cycle, and flavour group as factors showed a significant effect of soybean ( $F_{1,21} = 20.92$ ,  $P < 0.001$ ), cycle ( $F_{3,63} = 53.38$ ,  $P < 0.001$ ), soybean  $\times$  flavour group interaction ( $F_{1,21} = 14.27$ ,  $P < 0.01$ ), and soybean  $\times$  cycle interaction ( $F_{3,63} = 11.21$ ,  $P < 0.001$ ). Tukey's HSD tests confirmed that there was no soybean effect in cycles 1 or 2 ( $Q_{24,24} = 0.54$ ,  $P = 0.56$ ;  $Q_{24,24} = 0.92$ ,  $P = 0.39$ , respectively), and significant soybean effects in cycles 3 and 4 ( $Q_{24,24} = 7.34$ , and  $Q_{24,24} = 6.91$ , respectively; both  $P_s < 0.001$ ).

### 2.2.2. Revaluation phase: Pre-conditioning choice test, conditioned food aversion, and post-conditioning choice test

The day of the pre-conditioning choice test, a subject from group oregano became ill and was removed from the experiment; hence, analyses for this group were done with  $n = 11$ .

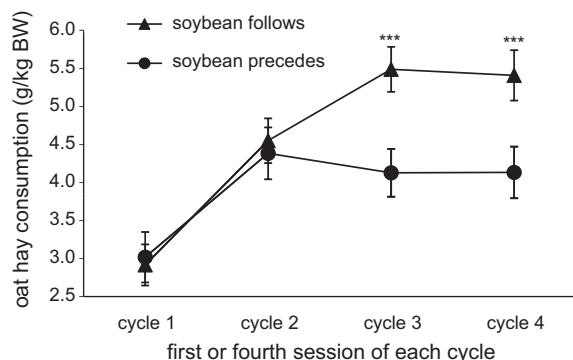




**Fig. 1.** Mean oat hay consumption across training sessions as a function of flavour group. The words “parsley” and “oregano” appearing above the data points indicate which oat hay flavour was served on those three sessions. For group parsley ( $n = 12$ ), parsley flavour was followed by 0.4% BW of soybean meal, and oregano flavour by an empty feeder in the second part of sessions. For group oregano ( $n = 12$ ), oregano flavour was followed by 0.4% BW of soybean meal, and parsley flavour by an empty feeder in the second part of sessions. Error bars denote  $\pm 1$  SE.

In the pre-conditioning choice test (pre-test in Fig. 3), subjects in group parsley preferred (i.e., ate more) parsley flavoured oat hay over oregano flavoured oat hay, whereas the opposite was true for subjects in group oregano. Mean ( $\pm 1$  SE) individual consumption of parsley flavour was 249 g ( $\pm 18$ ) in group parsley and 37 g ( $\pm 17$ ) in group oregano, whereas mean consumption of oregano flavour was 0 g in group parsley and 160 g ( $\pm 26$ ) in group oregano. A Mann–Whitney  $U$  test of the proportion of parsley flavour intake over the total consumption in the pre-test confirmed the significant difference between group parsley and group oregano ( $U_{12,11} = 0$ ,  $P < 0.001$ ). Fisher’s exact test of the frequency of subjects preferring parsley over oregano flavour as a function of flavour group was also highly significant (12 out of 12 subjects ate more parsley than oregano flavour in group parsley, whereas only 3 out of 11 subjects did so in group oregano;  $P < 0.001$ ).

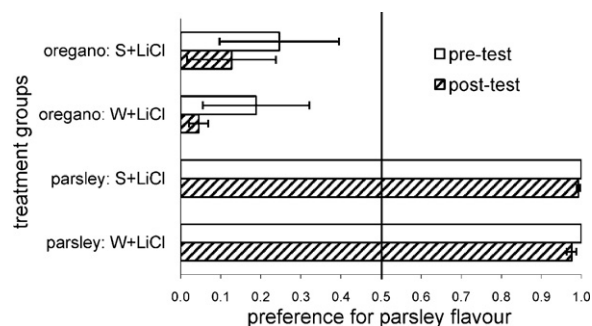
In the post-conditioning choice test (post-test in Fig. 3), mean ( $\pm 1$  SE) consumption of parsley flavour was 220 g ( $\pm 15$ ) for group parsley and 16 g ( $\pm 11$ ) for group oregano, whereas mean consumption of oregano flavour was 4 g ( $\pm 2$ ) for group parsley and 164 g ( $\pm 15$ ) for group oregano. A Mann–Whitney  $U$  test of the proportion of parsley flavour intake over the total consumption in the post-conditioning choice test confirmed the significant difference between group parsley and group oregano ( $U_{12,11} = 0$ ,  $P < 0.001$ ).



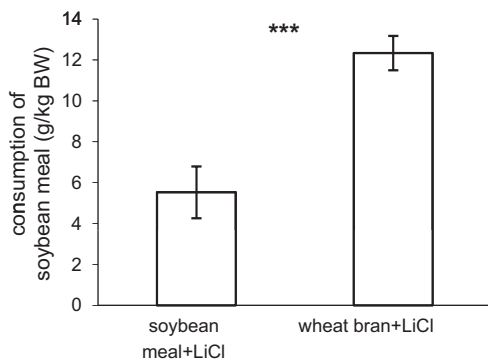
**Fig. 2.** Mean consumption of oat hay in the first or fourth session of each cycle as a function of whether soybean meal was accessed in the previous (soybean meal precedes oat hay access) or the current (soybean follows oat hay access) session ( $N = 24$ ). Error bars denote  $\pm 1$  SE. \*\*\* $P < 0.001$ .

Fisher’s exact test of the frequency of subjects preferring parsley over oregano flavour as a function of flavour group was also highly significant (12 out of 12 subjects in group parsley versus 1 out of 11 subjects in group oregano;  $P < 0.001$ ). In addition, the ANOVA of the proportion choice for the oat hay flavour followed by soybean meal during training (with flavour group, aversive conditioning treatment, and pre/post-conditioning choice test as factors) only showed a significant effect of flavour group ( $F_{1,19} = 9.12$ ,  $P < 0.01$ ), meaning that subjects from group parsley ate more parsley flavoured oat hay than the amount of oregano flavoured oat hay eaten by subject from group oregano (a fact that was also evident in the initial cycle of the training phase, and that suggests that the parsley flavour was more palatable than the oregano flavour; see Fig. 1). There was no effect of either pre/post-test (ANOVA:  $F < 1$ ,  $P = 0.39$ ), pre/post-test  $\times$  flavour group interaction ( $F_{1,19} = 1.49$ ,  $P < 0.24$ ), pre/post-test  $\times$  aversive conditioning interaction ( $F < 1$ ,  $P = 0.92$ ), or pre/post-test  $\times$  flavour group  $\times$  aversive conditioning interaction ( $F < 1$ ,  $P = 0.74$ ).

Last, those animals which had been intoxicated after soybean meal consumption ate significantly less soybean meal in a 5-min



**Fig. 3.** Preference for parsley flavoured (over oregano flavoured) oat hay before (pre-test) and after (post-test) the aversive conditioning session with LiCl. Preference was estimated as grams of parsley consumed over total intake in each choice session. For groups oregano ( $n = 11$ ), oregano flavoured oat hay was followed by soybean meal while parsley flavoured oat hay was followed by an empty feeder during training sessions; the opposite flavour-consequence pattern was set for subjects in groups parsley ( $n = 12$ ). For groups S + LiCl ( $n = 12$ ), subjects were intoxicated with lithium chloride (LiCl) after they ate soybean meal; whereas for groups W + LiCl ( $n = 11$ ), subjects were intoxicated with LiCl after they ate wheat bran. Error bars denote  $\pm 1$  SE.



**Fig. 4.** Mean consumption of soybean meal in a 5-min test as a function of aversive conditioning treatment. Three days before this test, subjects received an intra-ruminal infusion of lithium chloride (LiCl) immediately after they consumed soybean meal (i.e., group “soybean meal + LiCl”,  $n = 12$ ) or wheat bran (i.e., group “wheat bran + LiCl”,  $n = 11$ ). Error bars denote  $\pm 1$  SE. \*\*\* $P < 0.001$ .

test than those that had been administered LiCl after wheat bran consumption 3 days before (ANOVA:  $F_{1,19} = 17.66$ ,  $P < 0.001$ ; see Fig. 4). The effect of flavour group and the flavour group  $\times$  aversive conditioning interaction were not significant (both  $F_s < 1$ ,  $P = 0.91$ , and  $P = 0.74$ , respectively).

### 3. Discussion

Our results show an intake induction effect in sheep fed a low-nutritious feed in association with a high-nutritious supplement consistent with findings in other species (e.g., see Weatherly et al., 2005a,b, for induction effects in rats). Sheep learned to respond to oat hay as a function of whether its flavour was a predictor of a subsequent high-nutritious food (soybean meal) or an innocuous event (presentation of an empty feeder). After several training sessions, sheep increased intake of flavoured oat hay followed by soybean meal relative to flavoured oat hay followed by an empty feeder. Moreover, subjects preferred the flavour associated with the subsequent presentation of soybean meal over the alternative flavour in choice tests conducted once training had finished, i.e., when soybean meal was no longer offered. Finally, flavour preferences were unaffected by the devaluation of the soybean meal by means of pairing this high-quality food with gastrointestinal malaise. We discuss these findings next.

#### 3.1. Learning versus digestive state

When subjects were confronted with oat hay at the beginning of any training session, the main source of digestive/nutritional difference among subjects or across days within the same subject was whether they had eaten soybean meal the day before. From a nutritional perspective, access to soybean meal in a previous session could have stimulated oat hay consumption, because protein supplements enhance fibre digestion in the rumen (Matejovsky and Sanson, 1995; Sanson et al., 1990). However, our results do not support this digestive hypothesis as an explanation for intake induction. When food intake reached an asymptote across days, sheep ate more oat hay before than after having access to soybean meal, and differences in oat hay intake mainly responded to the experimental treatments (i.e., whether oat hay was followed by soybean meal or by an empty feeder). In other words, oat hay consumption was similar among sessions sharing the same treatment (either soybean or empty feeder); however, subjects sharply changed their level of consumption from one type to the other type of session, that is, when the treatment changed. All this strongly suggests that sheep learned to modulate oat hay consumption and preference as a function of the pairing between oat hay flavours

and distinctive subsequent events in training sessions. However, we found no evidence that previous access to soybean meal affected current oat hay consumption as we would have expected based on the digestive hypothesis.

#### 3.2. Associative mechanisms of induction and choice

In the introduction we referred to the distinction between the predictive and hedonic value of a particular food incentive (see references in Section 1.2). We initially wondered whether the induction effect on oat hay consumption would be the result of oat hay cues (e.g., its flavour) recalling the subsequent event in a session. If that was the case, altering the incentive and/or hedonic value of the high-nutritious meal, as we did with the aversive conditioning protocol, should have modified the subjects' response to flavoured oat hay. Our results show that the devaluation of the higher incentive (soybean meal) did not affect the subjects' response to the lower incentive (oat hay), as evidenced by their persisting preference for the flavour that was followed by soybean meal during training. Therefore, our data do not support the explanation of induction in terms of the predictive value of each particular flavour. This conclusion, however, needs a word of caution. Although sheep that were intoxicated after access to soybean meal showed a subsequent reduction in consumption of that food, they did not develop full aversion to this food. Full aversion would have implied complete rejection of the soybean meal after its pairing with the aversive effects of LiCl. However, animals ate some soybean meal after intoxication, although to a significant lesser extent than sheep which did not experience the association soybean meal-malaise. This is not surprising considering that previous work with sheep has shown difficulties at inducing strong and persistent aversions to familiar, nutritive foods (Burritt and Provenza, 1996). A stronger aversion to the preferred food would have improved the chances of detecting a potential re-evaluation of the oat hay flavour presumed to be associated with the soybean meal. This effect would have been reflected as a reduction in preference for the flavour previously paired with the devalued meal (e.g., see Delamater et al., 2006). In contrast, small fluctuations in flavour preferences from pre- to post-conditioning choice tests occurred in the opposite direction to what could have been predicted by the notion of changes in the signalling value of oat hay flavours. Thus, it is likely that the predictive value of the corresponding flavour was not responsible for the induction effect found in the present study.

An alternative explanation for the induction effect encompasses the hedonic hypothesis suggesting that the hedonic value acquired for each flavoured oat hay was affected by the training protocol. We propose that the process underlying the induction effect found in the present study was a consequence of the association between the corresponding flavoured oat hay and the post-ingestive consequences of the soybean meal. The misattribution of post-ingestive feedback between closely eaten meals could be the mechanism by which the hedonic value of a given flavour was enhanced as indicated by the subjects' relative consumption and preference. Similarly, it has been shown that sheep increase their consumption of and preference for low-nutritious or phytochemical-containing foods closely paired with meals of higher nutritional quality (Baraza et al., 2005; Villalba et al., 2006). Moreover, it has been shown that the close experience of different diets makes it difficult for the animal to discriminate among their post-ingestive consequences. For instance, goats could learn to associate three different plants species with negative, neutral, or positive post-ingestive consequences when each plant (and its specific post-ingestive consequence) was experienced in a different day. However, almost no discrimination was identifiable when the three plants were simultaneously available in a session, despite

arranging the corresponding post-ingestive feedback to match the level of consumption of each species (Duncan and Young, 2002).

#### 4. Conclusion

According to the present findings, consumption of a low-nutritious food (oat hay) was enhanced when access to oat hay occurred before access to a high-nutritious supplement (soybean meal) across days. In contrast, oat hay consumption seemed unaffected when access to oat hay occurred after access to the high-nutritious food in a previous session. Thus, we found no support to the hypothesis that intake induction in sheep may partially rely on nutrient supplementation. Moreover, intake induction did not seem to depend on the explicit anticipation of soybean meal. We therefore conclude that the most parsimonious explanation for intake induction in the present study is that sheep learned to value a specific flavoured oat hay based on its association with the post-ingestive effects of soybean meal which followed oat hay ingestion.

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