

TASTE PREFERENCES OF WEST AFRICAN DWARF EWES AT DIFFERENT TRIMESTER PERIODS

AKA, Lawrence Okonkwo

Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria.

Corresponding Author: Aka, L. O. Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.

Email: Lawrence_aka@yahoo.com **Phone:** +234 8134576173

ABSTRACT

Taste preferences of pregnant West African dwarf (WAD) ewes were studied at different trimester periods using the multiple choice preference test method. A total of twelve WAD ewes of average body weight of 15.96 ± 4.17 kg, divided into two groups (A and B) were used for the study. Six pregnant ewes (in group A) served as the experimental group while the remaining six non pregnant ones (in group B) served as the control group. Preferences for test solutions; sucrose (sweet), salt, 0.09 % quinine sulphate (QS, bitter), 10 % solution of sodium hydrogen trioxocarbodate (Na_2HCO_3 , sour) and glutamic acid (GA, umami) were investigated compared to water. The preference result showed that pregnant WAD ewes at early trimester had strongest mean preference for Na_2HCO_3 sour taste (84.31 ± 6.37 %) and moderate mean preference for (76.48 ± 6.11 %) for bitter taste compared to other test solutions that were either non discriminated or weakly preferred. At mid trimester, they showed moderate preference (71.15 ± 2.99 %) for sucrose which was highest compared to other test solutions that were either weakly preferred (60.08 ± 7.04 % quinine sulphate; 68.34 ± 3.86 % Na_2HCO_3), weakly rejected (45.13 ± 4.16 %, salt) or non discriminated (51.94 ± 6.14 %, GA). At late trimester, the preference for sucrose was strong and highest (89.43 ± 6.76 %) as well as GA (80.16 ± 4.95 %) compared to moderate preference for Na_2HCO_3 (76.14 ± 6.48 %), and rejection for salt (41.36 ± 5.73 %) and QS (28.14 ± 3.96 %). The results of this study have shown that pregnant WAD ewes respond differently to different tastants at different trimester periods. This information could serve as useful physiologic guide in appetite stimulation and probably explains the voluntary food selection behavior of the WAD ewes, during pregnancy.

Keywords: Taste, Pregnancy preference, WAD ewes, Trimester periods

INTRODUCTION

This study was prompted by the lack of data on taste responses in tropical ruminants particularly the West African dwarf sheep. No known quantitative information is particularly available for the pregnant WAD ewe. It is clear that accumulation of knowledge on this very rare area will result in a better understanding of the modulatory effect of pregnancy on taste responses and possible other biological mechanisms involved in taste phenomena in this tropical breed and sex of sheep under the

physiologic condition of pregnancy in the southern part of Nigeria. The ruminant animal is among the major supplier of animal protein in Nigeria and elsewhere in the world, thus any attempt towards understanding issues that boarder on nutrition and reproduction in general deserves commendation and much attention. Taste is a factor in nutrition that could be employed to enhance reproductive potentials of animals through its regulatory influence on nutrition (appetite, food palatability, acceptability and voluntary feed intake). Thus information on the taste responses of the WAD

sheep particularly during pregnancy would be a useful guide in the improvement of diet selection and food intake at all stages of pregnancy.

The problems usually encountered in the nutritional management of WAD sheep in general and the pregnant ewes in particular especially during the dry season (characterized by relative scarcity of feedstuffs) could be largely reduced if there is proper clarification on their taste preferences and its application to feeding practices under practical conditions. Good quantitative information on taste preferences by pregnant ewes might help to develop suitable diets that would be well accepted at different periods of pregnancy. The ability of pregnant animals to discriminate among different tastants and tastant concentrations has been reported (Nordin *et al.*, 2004). Such discrimination may be critical to food choices and reproductive performances during pregnancy.

Sensory perceptions at various points in the pregnancy of animals could be a serious factor regulating food intake in ruminants. Nordin *et al.* (2004) has reported increased smell, distorted smell, phantom smells and abnormal taste including increased bitter sensitivity and decreased salt sensitivity in pregnant women. Edmonds (2009) has reported changes in smell and taste perception at early and late stages of pregnancy in humans. Preferences for a variety of foods including sweets differ between males and females and during pregnancy (Duffy *et al.*, 1998; Prutkin *et al.*, 2000). Sex differences in food intake also have been reported in animal models (Tarttelin and Gorski, 1971; Wade, 1972) and reproductive hormones appear to be important in these differences (Krause *et al.*, 2003). Little work has examined trimester differences in taste preferences in animals. Hitherto, scientists have differed evidences for changes in taste during pregnancy and the origin of these changes. This work was aimed at understanding the influence of pregnancy on the taste responses of ruminants using the West African dwarf ewes.

MATERIALS AND METHODS

Animal Model: Twelve WAD adult ewes of average body weight 14.96 ± 4.15 kg purchased from Ibagwa market in Nsukka West Local Government, Enugu State, Nigeria were used for the study. They were quarantined for 21 days for observation for disease symptoms. Thereafter, they were transferred to a pen measuring $7.2 \times 5.3\text{m}^2$ in the animal house of the Department of Veterinary Physiology and Pharmacology. They were further kept for an adaptation period of 21 days during which they were fed combinations of *Panicum maximum*, *Stylosanthes gracilis* and *Pennisetum purpureum ad libitum*, with occasional supplementation of palm kernel cake, wheat and maize offal. Salt was provided as a lick once (24h) a week. 'Ecto' and 'endo' parasites were routinely controlled together with the prescribed vaccinations (Obidike *et al.*, 2009).

After 21 day adaptation period, oestrus synchronization was performed on six ewes (group A) utilizing a 100 mg progesterone (Longlife[®] China) in a single dose intramuscular injection (Ola and Egbuninke, 2005). Immediately upon administration of the progesterone, one (1) mature ram of proven fertility was introduced into the pen housing the group A ewes and estrus was monitored daily. The degree of estrous synchrony (%) within 9 hours of the first mating was recorded by visual observation - this took place between 2 to 7 days after estrus synchronization (Obidike *et al.*, 2009). Each time mating was observed, the mated ewes were identified with labeled tags tied around the neck, reflecting the animal number and the time of mating.

Taste: After two weeks, all ewes (groups A and B) were offered 10 liters of each solution of five different tastants in heavy metal containers in a multiple choice preference test methods (Goatcher and Church, 1970). Ten (10) liters of water was offered in a sixth container. Fresh preparations of solutions of the tastants were made daily thus: 2 % sucrose solution (sweet) (T1), 0.5 % salt solutions (salty) (T2), 0.09 % Quinine sulphate solution (bitter) (T3), 5 % sodium hydrogen trioxocarbonate solution

(sour) (T4) and 10 % glutamic acid solution (umami) (T5) (Kamalu *et al.*, 2006).

Daily consumption of each test solution was recorded after 24 hours of presentation to the ewes. The assumption was that percentage preference was directly related to percentage consumption which was given as follows: % consumption = Quantity offered – Quantity unconsumed / Quantity offered x 100.

The percentage preference chart (scores) was as follows: 10 – 20 % consumption indicated strong rejection, 21 – 30 % consumption indicated moderate rejection, 31 – 50 % consumption indicated weak rejection, 50 % consumption indicated the non-discriminatory zone (i.e. the test solution and water were equally consumed, 51 – 70 % consumption indicated a weak preference, 71 – 80 % consumption indicated a moderate preference and 81 – 100 % consumption indicated a strong preference (Goathcher and Church, 1970).

At 60 days post mating pregnancy was diagnosed and confirmed in all the six ewes in group a by the use of a portable Scanner (SN 5008, England). Therefore, pregnancy was confirmed to have been in place since the commenced of the experiment. Thus the trimesters of pregnancy were designated as follows: 0 – 50 days (early trimester), 51 – 100 days (mid trimester) and 101 – 155 days (late trimester).

Daily intake of the test solutions and water in both group A (pregnant) and B (non-pregnant) was recorded until parturition at 148 – 155 days.

Data Analysis: Data were recorded as percentage and analyzed using ANOVA, with the levels of probability set at 0.05. A mean difference of $p < 0.05$ in the preference for each test solution between any given trimester and control, and between trimesters were regarded as significantly different (Marija, 1993).

RESULTS

The results of this study demonstrated some classical variations in the taste preferences of pregnant and non-pregnant WAD ewes as well as between different trimester periods of

pregnancy. At early trimester (0-50 days) of pregnancy, there was moderate preference for Quinine sulphate (76.48 ± 6.11), non discriminatory response to sucrose (53.19 ± 7.41 %) and salt (55.26 ± 5.26 %), weak preference for glutamic acid (66.48 ± 4.28 %) and strong preference for 5 % solution of sodium hydrogen trioxocarbonate (84.31 ± 6.37 %) compared to the non-pregnant ewes that had strong preference for quinine sulphate (83.46 ± 4.61 %), moderate for glutamic acid (70.08 ± 5.46 %), moderate rejection for 5 % solution of sodium hydrogen trioxocarbonate (48.06 ± 4.66 %) and sucrose (46.93 ± 6.17 %) and weak preference for salt (68.59 ± 5.75 %) (Table 1).

At mid trimester (50-100 days) there was weak preference for Quinine sulphate (60.06 ± 7.04 %) and moderate preference for 5 % solution of sodium hydrogen trioxocarbonate (68.34 ± 3.86 %), moderate preference for sucrose (71.15 ± 2.99 %), weak rejection for salt (45.13 ± 4.61 %), non-discriminatory preference for glutamic acid (51.94 ± 6.14 %) (Table 2).

At late trimester (100-155 days) the ewes showed strong preference for sucrose (89.43 ± 6.76 %) and glutamic acid (80.16 ± 4.95 %), moderate preference for 5 % solution of sodium hydrogen trioxocarbonate (76.14 ± 6.48 %), moderate rejection for salt (41.36 ± 5.73 %), and strong rejection for quinine sulphate (24.14 ± 3.96 %) compared to the control (Table 3).

DISCUSSION

Anecdotal and empirical evidence in humans suggest that there are sex differences in food preferences and that reproductive hormones may be important in these differences (Kathleen *et al.*, 2005). There is evidence that during pregnancy women's sniffer and responses to taste change during menstrual cycle and at ovulation (Blezer *et al.*, 2009). Levels of estrogen during pregnancy have been indicated to be responsible for the heightened senses during pregnancy (Nordin *et al.*, 2004). This study has attempted to determine the taste responses of the West African dwarf ewes at

different trimester periods as to further relate the existing information on animals particularly the WAD ewes at various stages of pregnancy.

Table 1: Taste preference of pregnant WAD ewes at early trimester period

Test solution	Taste Preference (% consumption)	
	Early trimester	Non pregnant
T1	53.19±7.41	46.93±6.17
T2	55.26±5.26	68.59±5.75
T3	76.48±6.11	83.46±4.61
T4	84.31±6.37	48.06±4.66
T5	66.48±4.28	70.08±5.46
T0	48.19±6.33	50.63±4.11

T1, 10 % solution of sucrose (sweet), T2, 10 % salt solutions (salty), T3, 10 % solution of Quinine sulphate (bitter), T4, 10 % solution of sodium hydrogen trioxocarbonate (sour), T5, 10 % solution of glutamic acid (umami)

Table 2: Taste preference of pregnant WAD ewes at mid trimester period

Test solution	Taste Preference (% consumption)	
	Mid trimester	Non pregnant
T1	71.15±2.99	56.94±4.17
T2	45.13±4.61	64.49±5.73
T3	60.08±7.04	80.36±3.61
T4	68.34±3.86	55.66±6.63
T5	51.94±6.14	68.98±4.53
T0	50.96±6.75	49.63±2.82

T1, 10 % solution of sucrose (sweet), T2, 10 % salt solutions (salty), T3, 10 % solution of Quinine sulphate (bitter), T4, 10 % solution of sodium hydrogen trioxocarbonate (sour), T5, 10 % solution of glutamic acid (umami)

The observation at early trimester (0 – 50 days) show that a strong preference (84.31 ± 6.37 %) for 5 % solution of sodium hydrogen trioxocarbonate existed. This indicates that at early trimester the WAD ewes preferred bitter solution to all other test solutions.

Next to this was the moderate preference for Quinine sulphate solution (76.48 ± 6.11 %). These observations suggested that at early trimester the WAD ewes showed no aversion for bitter and sour solutions. It was also observed that during this period of pregnancy a non discriminatory response to

sucrose (53.19 ± 7.41 %) and salt (55.26 ± 5.26 %) as well as weak preference for glutamic acid (66.48 ± 4.28 %) occurred. We thought that estrogenic profile during pregnancy could have accounted for the observation made. Estrogen has been demonstrated to increase the taste threshold for sucrose in rats ((Kathleen *et al.*, 2005). It could therefore be that at early trimester, the serum estrogen level was high and thus lead to an increase in sucrose threshold and its consequent non discriminatory response.

The observed weak preference for glutamic acid suggests a probable aversion for high amino acid containing foods during early trimester.

At mid trimester (50 – 100 days), the preference for quinine sulphate became weaker compared to the early trimester as it decreased from 76.48 ± 6.11 % to 60.06 ± 7.04 % at early and mid trimester respectively. This created a picture that bitter tastants are detested at mid trimester. This scenario was also applicable to 10 % solution of sodium hydrogen trioxocarbonate (sour) with weak preference value of 68.34 ± 3.86 % at mid trimester. The relative avoidance of bitter and sour tastants at mid trimester period compared to the early trimester suggest that at this time of pregnancy ewes adopt a physiologic adjustment to avoid consumption of plant materials containing high levels of bitters and sour substances and in so doing protect the developing fetus (es) from the possible teratogenic effects of such compounds contained in plant materials. It is known that most bitter and sour substances contained in most plants are biologically active compounds that when taken in excess are capable of exerting harmful effects to either the dam or most importantly the fetus (es). Therefore, it could be that the relatively increased aversion for quinine sulphate and Na₂HCO₃ at mid trimester was a behavioral response to protect the fetus (es) from such possible harmful effects during this second half of pregnancy with greatest increase in fetal growth. Also at this period, sucrose was most highly and moderately preferred (71.15 ± 2.99 %) compared to other test solutions while salt was weakly rejected

(45.13 ± 4.61 %). The fact that sucrose mostly preferred in the present circumstance probably was to meet the energy demands placed on the dam by the developing fetus (es).

Table 3: Taste preference of pregnant WAD ewes at late trimester period

Test solution	Taste Preference (% consumption)	
	Late trimester	Non pregnant
T1	89.43±6.76	48.98±4.27
T2	41.36±5.73	63.39±3.25
T3	24.14±3.96	81.43±3.71
T4	76.14±6.48	50.46±3.22
T5	80.16±4.95	66.18±3.96
T0	45.68±5.33	53.63±3.14

T1, 10 % solution of sucrose (sweet), T2, 10 % salt solutions (salty), T3, 10 % solution of Quinine sulphate (bitter), T4, 10 % solution of sodium hydrogen trioxocarbonate (sour), T5, 10 % solution of glutamic acid (umami)

Table 4: Statistical comparison of taste preferences at different trimester periods in pregnant ewes

Test solution	Taste Preference (% consumption)		
	Early trimester	Mid trimester	Late trimester
T1	^a 53.19±7.41 ^a	^a 71.15±2.99 ^b	^a 89.43±6.76 ^c
T2	^a 55.26±5.26 ^b	^b 45.13±4.61 ^a	^b 41.36±5.73 ^a
T3	^b 76.48±6.11 ^a	^{e,c} 60.08±7.04 ^a	^c 24.14±3.96 ^c
T4	^b 84.31±6.37 ^a	^{c,a} 68.34±3.86 ^b	^d 76.14±6.48 ^a
T5	^c 66.48±4.28 ^a	^{e,d} 51.94±6.14 ^b	^a 80.16±4.95 ^c

a, b, c, d and e = left superscript indicate comparisons of preferences within trimester, while right superscript indicate comparisons of preferences between trimester. Values with same superscript (left or right) are not significantly different at a probability of 0.05. T1, 10 % solution of sucrose (sweet), T2, 10 % salt solutions (salty), T3, 10 % solution of Quinine sulphate (bitter), T4, 10 % solution of sodium hydrogen trioxocarbonate (sour), T5, 10 % solution of glutamic acid (umami)

Energy is provided to the fetus mainly in the form of glucose; however, amino acids as well as the water soluble vitamins and steroids serve as energy sources and are supplied across the placenta. If the energy supply during this phase of pregnancy become inadequate, pregnancy toxemia or twin lamb disease likely results just before parturition especially in ewes that are carrying more than one fetus. The moderate preference for sucrose could have been a way

of meeting the energy needs which was expected to be high at mid trimester. More so, it has been reported that in a variety of mammalian species, sucrose test fibers of the chorda tympani nerve are easily excited (Ninomiya and Funakoshi, 1993; Danilova *et al.*, 1997). It is however difficult to explain why the hyper excitability of the nerves was more pronounced during the mid trimester in this study. The weak rejection of salt solution probably was to maintain the normal osmotic balance of the body fluids and fetal fluids (chorionic and allantoic) during the first and second halves of pregnancy. According to Thompson (2004) relative changes in uterine contents during pregnancy are first an increase in fetal membranes, followed by a large increase in fetal fluids during the first two phases of pregnancy.

At late trimester (100 – 155 days), the preference for sucrose (89.43 ± 6.76 %) and glutamic acid (80.16 ± 4.95 %) became stronger which indicated further adjustments for meeting energy demands in readiness for parturition. If the finding that estrogen increased the threshold for sucrose (Kathleen *et al.*, 2005) is anything to go by and if circulating estrogen concentration is usually much elevated only towards the end of pregnancy in the cow, ewe and sow (Thompson, 2004), it becomes difficult to associate the strong preference for sucrose at late trimester with plasma estrogenic concentration. As such, other mechanism(s) apart from estrogenic concentration could be responsible. Also at late trimester

the moderate preference for 10 % solution of sodium hydrogen trioxocarbonate (76.14 ± 6.48 %), moderate rejection for salt (41.36 ± 5.73 %), and strong rejection for quinine sulphate (24.14 ± 3.96 %) compared to the control probably was a hormonal effect. These observations at every stage of pregnancy could have been occasioned by either estrogenic or progesteronic effect on the peripheral components of the gustatory system. However,

the effect estrogen on lingual taste receptors has been minimally examined. We are speculating that there could be changes in the number of amiloride-sensitive sodium (Na) channels in taste receptors, as well as the responsiveness of isolated taste receptors cells of the WAD ewes at various stages of pregnancy. Also there could be hormonal effects on gustatory nerves such as the whole-nerve chorda tympani nerve and greater superficial petrosal nerve that carry sweet taste information to the central nervous system. Different sensory nerves are involved in detecting the tastes of different solutions in different animals (Smith and Frank, 1993) and possibly under different physiologic conditions such as pregnancy or its phases in the same animal. An animal's response to different taste solutions is also based on the diversity of the chemical structure of solutions and the interpretation of genetic physiological and behavioral attributes (Froloff *et al.*, 1996). Also, transduction of the basic taste is thought to involve multiple mechanisms specific for compounds with distinct structural features (Walters *et al.*, 1991). These factors probably account for the variations in the taste responses during these phases of pregnancy. Likewise, hunger has been shown to modify taste (Goathcher and Church, 1970; Overman, 1980), but this could not have been a contributory factor in this study as feeding was adequate for the entire experimental period.

In conclusion, this study has demonstrated that there are variations in the taste preferences of the WAD ewes at different trimester periods but at this moment, we cannot clearly identify the mechanism(s) responsible for these variations. It is thought that some degree of hormonal influence could be responsible and that such variations could be necessary for homeostatic adjustments and food selection. These characteristic taste responses in pregnant ewes could therefore be exploited in formulating suitable, palatable and acceptable diets for the WAD ewes at any given stage of pregnancy.

REFERENCES

- BELZER, L. M., SMULIAN, J. C., LU, S. E. and TEPPER, B. J. (2009). Changes in sweet taste across pregnancy in mild gestational diabetes mellitus: Relationship to endocrine factors. *Chemistry of Senses*, 34(7): 595 – 603.
- BROWN, J. E. and TOMA, R. B. (1986). Taste changes during pregnancy. *American Journal of Clinical Nutrition*, 43: 414 – 418.
- CARRUTH, B. R. and SKINNER, J. D. (1992). What do pregnant adolescents believe about nutrition during pregnancy? *Family and Consumer Sciences Research Journal*, 21(1): 22 – 33.
- DANILOVA, V., HELLEKANK, G., ROBERTS, T., TINT, J. M. and NOTRE, C. (1997). Behavioral and single chorda tympani taste fiber responses in common marmoset, *Callithrix jacchus*. *Chemistry of Senses*, 22: 225 – 238.
- DUFFY, V. B., BARTOSHUK, L., STRIEGELMOORE, L. and RODIN, J. (1998). Taste changes across pregnancy. *Annals of New York Academy of Science*, 855: 805 – 809.
- EDMONDS, M. (2009). How does pregnancy affect senses of smell and taste? <http://health.howstuffworks.com/pregnancy.affect-sense-fo-smell-and-taste-1.htm> Date assessed: December 14, 2009.
- FROLOFF, N., FAURION, A. and MACLOAD, P. (1996). Multiple human taste receptors: a molecular modeling approach. *Chemistry of Senses*, 21: 425 – 445.
- GOATHCHER, W. D and CHURCH, D. C. (1970). Taste responses in ruminants. 1. Reactions of sheep to sugars, saccharin, ethanol and salts. *Journal of Animal Science*, 30: 777 – 783.
- KATHLEEN, S., STRATFORD, J. M. and CONTRERAS, R. J. (2005). Estrogen increases the taste threshold for sucrose in rats. *Physiology and Behavior*, 86: 281 – 286.

- KAMALU, T. N., AKA, L. O., OBIDIKE, R. I. and AGU, D. C. (2006). An investigation into the taste sensitivity and modulation of fasted, non-fasted, insulin injected and non-insulin injected pigs. *Nigeria Journal of Applied and Experimental Biology*, 7: 43 – 48.
- KENNEDY, J. M. and BALDWIN, B. A. (1972). Taste preferences in pigs for nutritive and non-nutritive sweet solutions. *Animal Behavior*, 20: 706 – 718.
- KRAUSE, E. G., CURTIS, K. S., DAVIS, L. M., STOWE, J. R. and CONTRERAS, R. J. (2003). Estrogen influence stimulates water intake by ovariectomized female rats. *Physiology and Behavior*, 79: 267 – 274.
- MARIJA, N. (1993). One way analysis of variance, *SPSS for Windows Base Systems User's Version 11.0*. Illinois, Chicago.
- NINOMIYA, Y. and FUNAKOSHI, M. (1993). Genetics and neurobehavioral approaches to the taste receptor mechanism in mammals. Pages 253 – 272. *In: SIMON, S. A. and ROPER, S. D. (Eds). Mechanism of Taste Transduction*. CRC Press, Boca Raton, Florida, USA.
- NORDIN, S., BROMAN, B., OLOFSSON, J. K. and WULFF, M. (2004). A longitudinal descriptive study of self-reported abnormal smell and taste perception in pregnant women. *Chemistry of Senses*, 29(5): 391 – 402.
- OBIDIKE, I. R., AKA, L. O. and OKAFOR, C. I. (2009). Time-dependant peri-partum haematological, biochemical and rectal temperature changes in West African dwarf ewes. *Small Ruminant Research*, 82(1): 53 – 57.
- OLA, S. and EGBUNIKE, G. N. (2005). Estrous synchronization with progestrogen injectables in West African dwarf does. *Nigerian Journal of Animal Production*, 1: 126 – 133.
- OVERMAN, S. R. (1980). Feeding Behavior. Pages 414 – 435. *In: DENNY, M. R. (Ed). Comparative Psychology, An Evolutionary Analysis of Animal Behavior*. John Wiley and Sons, New York.
- PRUTKIN, J., DUFFY, V. B., ETTER, L., FAST, K., GARDNER, E. and LUCCHINA, L. A. (2000). Genetic variation and inferences about perceived taste intensity in mice. *Physiology and Behavior*, 69: 161 – 173.
- SMITH, O. A. and FRANK, M. E. (1993). Sensory coding by peripheral taste fibers. Pages 295 – 338. *In: SIMONS, S. A. and ROPER, S. D. (Eds). Mechanism of Taste Transduction*. CRC Press, Boca Raton, Florida, USA.
- TARTTELIN, M. F. and GORSKI, R. F. (1971). Variations in food and water intake in the normal and acyclic female rats. *Physiology and Behavior*, 7: 847 – 852.
- THOMPSON, F. N. (2004). *Female Reproduction in mammals*. Pages 711 – 712. *In: WILLIAM, O. R. (Ed). Duke's Physiology of Domestic Animals*, 12th edition, Cornell University Press, London.
- WADE, G. N. (1972). Gonadal hormones and behavioral regulation of body weight. *Physiology and Behavior*, 8: 523 – 534.
- WALTERS, D. E., ORTHOETER, F. T. and DUBOIS, G. E. (1991). *Sweetness, Discovery, Molecular Design and Chemoreception*. ACS, Washington DC, USA.