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Phenotypical and metabolical–induced variation of meat nutritive value within the same variety of Roman snail (*Helix pomatia* var. *Banatica*)

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Abstract

Inter- and intra-specific variations of snail meat nutritive value are well documented. The same may be true even within the same species variety. Both active and hibernating snails were collected from two distinct sites from geographical and subclimatic point of view. Using bidimensional data processing, statistical, and biochemical analyses we assessed the actions of environmental factors and metabolical status on intra-variety changes of aperture morphological features and foot meat chemical composition. We demonstrated that aperture area tended to be larger in wetter areals whereas relationships between aperture height and foot weight sugested the lack of scientific background for calibration of wild snails. As regards the influence of metabolic status and location on foot chemical composition our results pointed to raw protein as possible predictor of genetic and environmental-induced biochemical variations within the same species variety while percentage content of raw protein in the total amount of dry matter displayed, regardless of origin, a relatively constant variation in relation to the metabolic status.

Keywords: Roman snail, *Helix pomatia*, phenotype, snail meat

1. Introduction

From evolutionary point of view, the gastropods are one of the most versatile group of mollusks, characterized by an extraordinary biodiversity and an amazing capacity to adapt to various environmental conditions [1]. Since snails were abundant and easily available, they have been used in human nutrition since the Neolithic Age [2]. The snail meat was highly priced in the Roman Empire as a culinary delicacy [3] whereas its use in various medicinal purposes was mentioned since Antiquity [4]. Nowadays consumption of snail meat is quickly increasing, based especially on its high nutritive value and small amount of fat [5,6].

The energy value of snail meat is 67 kcal/100g, much lower than of the leanest meat or fish [7]. Recent studies also revealed increased amounts of lipid rich in essential (e.g., linoleic acids) and polyunsaturated fatty acids (e.g., palmitic acid, estearic acid, arachidonic acid) with more than 20 carbon atoms in snail` meat [8]. In addition, it represents an important source of essential minerals such as Ca, P, K, Mg, Na, Fe, Mn, and Zn [9]. As a result, snails can represent an alternative food for people with high protein and low fat requirements [10]. Among many species of edible snails the Roman snail (*Helix pomatia*), also called the burgundy snail, is one of the most expensive and appreciated on the international market [11].

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The price per one kilogram of processed meat ranges on the EU market from four up to ten € [12]. Although in the last decades in many European countries specialized farms were set up aiming to rear them in controlled environments [13] the main part of the increasing demand from the international market is still meet by gathering them from the wild [14]. Before the winter arrival, the Roman snail (*Helix pomatia*) burrows themself and a thick, ferm, and compact epiphragm is formed over aperture [15,16] The hibernation is a metabolic status characterized by the vital functions decrease to the subsistence level [17]. As a result, the digestive traffic is stopped, the body moisture decreases, whereas the fat reserves are stored in the digestive glands[18].

This passive status provides the live Roman snails the longest preservation (over four months) whereas their economical value is the highest [13]. Prior to their processing the snails are calibrated on different categories as regards their economical value [19]. The aperture height, estimated as the major diameter of the ellipse circumscribed to the shell aperture, is used as guiding mark in this process [20].

However, there is little information to support this assumption in the case of snails gathered from the wild. In addition, genetically determined variations in snail conchological parameters are well known [21,22]. As a result, we investigated the scientific base of this process in respect to the moment of harvesting.

On the other hand, albeit several studies [9,23-26] reported small differences as regards biochemical composition of *H. pomatia* meat there are no relevant information in connection with the influence of local climatic conditions. Contextually, the enlightment of the scientifical base of these variations within the same species variety may provide an objective overlook on relationships between phenotype and genotype from bioproductive point of view (e.g. meat chemical composition, foot weight, processing efficiency).

2.Material and Method

Snails gathering. Snails were systematically classified according to information provided by the branch literature in respect to the shell shape, appearance, and color: *Helix pomatia* var. *Banatica* Kimakowicz, 1890 [1]. When the snails reach adulthood the aperture lip becomes hardened and turned-out whereas the only conchological feature that continues to increase is the shell thickness. As a result, the variability of live snail weight is almost entirely associated to body weight.

The locations and the moment of harvesting were chosen aiming to assess the influence of distance (about 100 km) and metabolic status on the variability of bioproductive features within the same subspecies of *Helix pomatia*. Thus, both active and hibernating snails were gathered for each of the two locations: Oraviţa (Caraş-Severin county; Latitude: 45.20°N; Longitude: 21.41°E) and Green Forest, nearby Timişoara (Timiş county; Latitude: 45.47°N; Longitude: 21.17°E) as see in Table 1.

Table 1. The samples area of origin and metabolic status

Sample	Area of origin	Metabolic status	Monthly mean rainfall level (mm)	Annual average temperature (°C)	Altitude (m)
OH	Oraviţa	hibernation	74.58 ± 17.68	11.8	309
OA	Oraviţa	activity	**Temperate continental climate**		
GA	Green Forest	activity	56.07 ± 11.89	10.6	91
GH	Green Forest	hibernation	**Temperate continental climate with Mediterranean influences**		

Table 2. Shell biometric features means, ranges and variability coefficients (n = 20)

Area of origin	**Oraviţa area (OH, OA)**			**Green Forest area (GH, GA)**		
Biometric parameter	**X ± SE**	**Range**	**CV**	**X ± SE**	**Range**	**CV**
Aperture width (mm)	23.21 ± 2.64	19.00 – 28.50	11.12%	20.01 ± 2.56	14.80 – 24.60	12.50%
Aperture height (mm)	27.36 ± 1.68	24.60 – 31.20	6.05%	25.24 ± 2.67	20.10 – 30.80	10.30%
Aperture area (cm^2)	2.00 ± 0.32	1.46 – 2.71	16.05%	1.60 ± 0.12	0.93 – 2.28	21.34%

Table 3. Bioproductive features means, ranges, and variability coefficients for the four samples (n = 10)

Biometric parameter	**X ± SE**	**Range**	**CV**	**X ± SE**	**Range**	**CV**
Sample	**OH snails**			**OA snails**		
Snail weight (g)	16.29 ± 4.79	10.82 – 26.70	27.80%	22.83 ± 2.99	15.90 – 26.89	12.41%
Foot weigth (g)	3.15 ± 0.73	1.62 – 4.08	21.95%	3.91 ± 1.55	2.48 – 7.32	37.50%
Slaughtering efficiency (%)	20.15 ± 5.00	10.17 – 26.74	23.40%	16.95 ± 5.19	10.28 – 27.13	28.90%
Sample	**GH snails**			**GA snails**		
Snail weight (g)	14.86 ± 3.83	10.54 – 24.38	24.30%	18.41 ± 4.72	13.81 – 20.83	11.10%
Foot weigth (g)	3.28 ± 0.81	2.55 – 5.35	23.30%	3.63 ± 0.85	2.12 – 4.93	22.05%
Slaughtering efficiency (%)	22.38 ± 3.47	18.21 – 29.11	14.60%	20.36 ± 6.68	10.20 – 30.92	30.91%

Table 4. Snail`s meat chemical composition and nutritive paramters for the four samples (n = 10).

Chemical composition	**OH**	**GH**	**OA**	**GA**
H_2O%	83.33%	81.54%	83.90%	82.82%
RP%	13.09%	13.96%	13.01%	14.02%
RC%	1.29%	1.15%	1.25%	1.05%
RF%	1.75%	1.89%	1.49%	1.28%
SEN%	0.54%	1.48%	0.36%	0.83%
Moisture:dry matter ratio				
H_2O%	83.33%	81.54%	83.90%	82.82%
DM%	16.67%	18.47%	16.10%	17.18%
Raw protein:dry matter ratio (RP/DM)				
RP(%)	78.52%	75.60%	80.76%	81.62%
OS(%)	21.48%	24.40%	19.24%	18.38%
Raw fat:dry matter ratio (RF/DM)				
RF(%)	7.74%	6.20%	7.79%	6.09%
OS(%)	92.26%	93.80%	92.21%	93.91%

Legend: H_2O%, moisture content (%); RP%, raw protein content (%); RC%, raw cellulose content(%); RF%, raw fat content (%); SEN%, nitrogen free extract (%); RP(%), percentage of raw protein in total dry matter content (%); RF(%), percentage of raw fat in total dry matter content (%), OS(%), percentage of other substances in total dry matter content (%).

In the Oraviţa area the snails were gathered from a vegetable garden (samples OH and OA) whereas in the Timisoara area they were collected from the Green Forest edge, near E70 European road (samples GH and GA).

Because the soil horizons were not planar in both locations, but undulated, with bumpy terrain (wooded land in the Green Forest area), the Roman snails (*Helix pomatia*) were sampled, from an area of 100 sqm using the random-walk technique [27].

Choosing the anesthesia method. Usually, the snails are anesthetezied using a solution made up of ¼ ethanol and ¾ distilled water . Since alcohol induces protein precipitation and meat dehydration [28] the meat changes its consistency, from juicy and soft to crusty and rigid this process might also affect the chemical composition of snail meat. As a result, snails were drowned by introducing them into a recipient filled with water for 24 hours.

It must be mentioned that prior anesthesia, all hibernating snails were first awaken by removing the aperture epiphragm and then, they were introduced in a recipient filled with lukewarm water (tºC = 24ºC).

*Determination of biometric features.*The aperture biometric features (aperture height, aperture width) were established using a vernier caliper [29]. Thus, the shell was positioned with the apex upwards and the aperture up to the operator. Next, the aperture height and width were estimated as the major and the minor diameter of the ellipse that circumscribes the aperture. To provide accurate results, each measurement was repeated for three times and only the mean value was taken into account. Finally, the aperture area was estimated as the ellipse circumscribed by the aperture width and depth according to the formula:

$$A_a = \pi a_a b_a / 1000$$

where A_a = aperture area (cm^3), a_a = aperture height (mm) and b_a = aperture width (mm).

*Assessment of processing efficiency (η%).*Snail were washed and then weighted by using an analytical balance PCE-AB 100. Because only the Roman snail`s (*Helix pomatia*) foot is usually used in human nutrition, the pedal mass was separated through a longitudinal incision performed on the joint between the visceral mass and the snail`s body. The slaughtering efficiency was estimated as percentage ratio (%) between the foot and the snail weight.

Determination of foot chemical composition . After killing the snails their meat was hermetically stored in closed and labeled containers and then it was frozen to -10°C until analysis. For each sample, the foot meat was minced in an electric blender. Next, all samples were analyzed to determine the meat chemical composition. The moisture level (H_2O%) was assessed by meat desiccation, the raw protein (RP%) by Kjedahl method, the ash content (AC%) by sample calcination, and the raw fat (RF%) by Soxhlet method.

Climatic data. Information related to the climatic particularities of the two sampling areas (Table 1) were collected from the literature [30,31] and from the historical meteorological database available on-line [32]. The following parameters were taken into account: subtype of temperate climate, annual average temperature (AAT), annual rainfall level (AAR), and monthly average rainfall (MAR) from 1995 to 2005 and altitude. Only MAR variation was estimated by statistical analysis, whereas the other factors were used as mean values, to understand if they can provide a reliable base for future studies about the influence of climatic factors on shell morphology.

Statiscal analysis. Biometric and biochemical parameters analysis was based on the descriptive, parametric, and nonparametric tests available on line at http://faculty.vassar.edu/lowry/VassarStats.html. First, the distribution normality was established for aperture height and width (Z test, two-tailed, df = 2, 36). Next, a simple correlation analysis (Pearson correlation) was conducted to quantify the relationships between aperture height and width. Then, we determined the variations of these biometric features between the two snail populations (F test, two-tailed, df = 2, 36).

After that, we assessed the origin influence on the aperture height, width, and area (T test, one-tailed, df = 2, 36). Second, the annual rainfall levels were analyzed regarding their distribution normality (Z test, two-tailed, df = 2, 116). Then, we estimated the differences between the two habitats by using the Mann-Whitney test (two-tailed, df = 2, 22).

Third, it was established the influence of origin and metabolic status on the snail bioproductive features: whole body weight (including shell) and foot weight. Next, it was estimated the distribution normality (Z test, two-tailed, df = 2, 18). The influence of origin on these features was assessed by the Mann-Whitney test (two-tailed, df = 2, 18) and the influence of metabolic status with the Wilcoxon test (two-tailed, df = 2, 18). The correlations among aperture height, snail`s and foot weight were established by nonparametric analysis (Spearman correlations).

Finally, it was determined the influence of metabolic status and origin on chemical composition (H_2O:DM ratio) of the foot meat by using Chi-square test (χ^2 test, df = 1, two-tailed). To enable an acurate determination of raw protein (RP) and fat (RF) variation in relation with origin and metabolic status, it was calculated their procentual ratio (%) in the total amount of dry matter (DM) as RP/DM (%) and RF/DM (%), respectively. These parameters were noted as RP(%) and RF(%). After that, the influence of metabolic status and origin on them was estimated using again χ^2 test (df = 1, two-tailed). Data are presented as mean values including standard errors (X ± SE). A p-value <0.05 was considered significant.

3.Results and discussion

Intra-variety changes of aperture parameters. Descriptive statistics revealed (Table 2) that, as regards the aperture height, the snails living in the Oraviţa area were more homogeneous than their counterparts from the Green Forest area whereas the aperture width presented a medium-sized variability irrespective of location. The Z test proved a normal distribution for all the biometric parameters taken into account (at least $P < 0.05$). In addition, the shell aperture was significantly distinct wider ($t = 2.88$, $P < 0.01$) and very significantly higher ($t = 3.79$, $P < 0.001$) for the same population. Furthermore, there were strong correlations between the aperture height and width in the Oraviţa area ($R = 0.71$, $P < 0.0001$) as well as in the Green Forest area ($R = 0.77$, $P < 0.0001$).

On the other hand, F test displayed no significant differences in respect to aperture biometric features for both *H. pomatia* populations: aperture height (F = 0.50, P = 0.16), aperture depth (F = 1.06, P = 0.89), aperture area (F = 0.84, P = 0.72). As a result, the aperture area was very significantly higher (t = 3.59, P = 0.001) for snails inhabiting the Oraviţa area (OH, OA).

Following land snails` low mobility, the shell`s biometric features tends to reflect geographical variation of environment [33]. This is ussualy matherialized in changes of ratio shell height/shell diameter [21] and in some cases, by alteration of aperture parameters [34]. Our data pointed that these variations might also occur in the case of snails that belong to the same species and variety, but live in different areas. Thus higher aperture height/width ratios were found for GH and GA snails (1.25 vs. 1.17).

The annual rainfall level presented a higher monthly variability in the Oraviţa area (Table 1). Z test proved an abnormal distribution for this parameter, regardless of location (P > 0.05). In addition, significant differences were revealed between the monthly rainfall level (MMA) registered in both areals (U = 13.00, P = 0.001). Relative aperture area tends to be smaller under drier conditions probably because of selection for smaller whorl cross-sectional area to reduce water loss [35]. Although our results support this assumption, most probable they are associated to cumulated actions of various environmental factors (e.g. meteorological conditions, soil composition, pollution degree, anthropization level, altitude, etc.) than to the phenotypic impact of a single one.

Intra-variety changes of biochemical parameters . Descriptive statistics showed a lower variability of snail weight (Table 3) for the active snails (OA, GA) than for their hibernating counterparts (OH, GH). Furthermore, Z test demonstrated that the snail weight was normally distributed for OA (P < 0.05) and GH (P < 0.01) snails whereas for OH and GA samples these values were abnormally distributed (P > 0.05). On the other hand, the foot weight presented at least a medium sized variability (Table 2) and was abnormally distributed (P > 0.05). Nevertheless, irrespective of metabolic status and origin, there were no correlations between the aperture height and the foot weight: OH (Rs = 0.08); OA (Rs = 0.02); GH (Rs = 0.01); GA (Rs = 0.07).

These results are in concordance with the fact that once the snails reach adulthood the shell grows only in thickness [36] and as a result, it is expected that the metabolic status will have no influence on the variation of aperture biometric features.

On these lines, although snails` calibration is still considered an useful tooll that allows the selective gathering of only the mature *H. pomatia* from the wild the latter data raise serious questions about the scientific base of this process as a reliable indicator of their economical value prior to industrial processing. As recent studies suggested [14] we assume that the establishment of well-organized systems for monitoring wild populations and the development of specialized rearing facilities may represent a more efficient alternative to the use of calibration as the main base to control the gathering of wild Roman snails.

Although the processing efficiency ranges between 10.17% and 30.92% (Table 3), it fitted widely to the interval considered standard for this species: 20-30 % [37]. According to Mann-Whitney test for comparing two unmatched samples, the area of origin displayed a distinct influence on the snail`s weight during hibernation (OH vs. GH: U = 73.00, P = 0.003) and no influence on the active ones (OA vs. GA: U = 50.00, P = 0.436). Oppositely, Wilcoxon test for related samples showed that the metabolic status induces distinct differences in respect to snail`s weight in both locations: OH vs. OA (W = 43.00, P = 0.012) and GH vs. GA (W = 47.00, P = 0.027). According to Mann-Whitney test for comparing two unmatched samples, the origin had no significant influence on the foot weight: OH vs. GH (U = 46.00, P = 0.65) and OA vs. GA (U = 46.50, P = 0.62). Similarly, according to Wilcoxon test for related samples, the metabolic status did not influence the foot weight, regardless of origin: OH vs. OA (W = 36.00, P = 0.13) and GH vs. GA snails (W = 13.00, P = 0.28). Generally, features with low variability represent more reliable and subtle predictors of genetic and environmental-induced variations within the same species variety than the ones with higher variability. As a result, we analysed the foot chemical composition and not the whole body one.

Since protein are essentially for human growth [38] one main feature used to assess the snail`s meat nutritive quality is the RP(%). According to France National Food Database, the chemical composition of *Helix pomatia* meat includes 79% moisture and 16% raw protein content [10].

Generally, higher values are mentioned for the snails breed in specialized farms [5,39] as compared to the wild ones [7]: 15.50-17.25% vs. 13.40-16.33%. Nonetheless, several exceptions were reported such as the wild *Helix pomatia* collected from the Cukurova region in Turkey whose RP(%) reached 18% [9]. The metabolic status exhibited a significant influence on the ratio between the foot moisture and dry mater content (RP/DM), regardless of origin: OH vs. OA snails (χ^2 = 0.01, P = 0.009) and GH vs. GA snails (χ^2 = 0.06, P = 0.008). In contrast, the origin induced distinct differences on the foot chemical composition between OH and GH snails (χ2 = 0.11, P = 0.007) and no differences in the case of OA and GA snails (χ^2 = 0.04, P = 1). Regardless of metabolic status, RP/DM ratio was significantly influenced by the area of origin: OA vs. GA (χ^2 = 0.94, P = 0.021) and OH vs. GH (χ^2 = 0.24, P = 0.006). Oppositely, the metabolic status displayed a distinct influence on RP/DM ratio for the snails living in the Oraviţa area (OH vs. OA: χ^2 = 0.16, P = 0.007) and no influence on their counterparts from the Green Forest area (GH vs. GA: χ^2 = 3.33, P = 0.14). On the other hand, the origin and the metabolic status had no significant influence on the RF/DM ratio: OH vs. GH (χ2 = 0.09, P = 1), OA vs. GA (χ2 = 0.44, P = 1), OH vs. OA (χ2 = 0.03, P = 1) and GH vs. GA (χ2 = 0.49, P = 1). Thus we concluded that foot RP(%) can rather be used as possible indicator of variability within the same variety of a species than RF(%).Our results showed that variations of weight induced by both the area of origin and the metabolic status were limited only to the live snails` weight. In both populations the meat humidity (RM) was always higher for the active snails (OA, GA) whereas the raw protein (RP), the dry matter (DM), and the fat content (RF) were lower (Table 4). Overall, these data are consistent with the fact that – before winter arrival – snail metabolic activity slows down to the subsistence level [40] whereas the soft tissues water content decreases as compared to the active status [40,41]. If the snail`s weight is estimated by the formula:

$$G_s = G_f + G_v + G_s$$

where G_s= live snail weight (g). G_f = foot weight (g). G_v = visceral mass weight (g), and G_s = shell weight (g) it is obvious why during hibernation the moisture (RM) decreases especially in visceral mass, whereas the weight loss and the processing efficiency are higher on hibernating snails.

We have also suggested that different RP/DM ratio in relation to the same metabolic status could be related to climate variations. On the one hand, milder winters, higher AAR and AAT stimulate plant development and metabolic activity and on the other hand, induce a shorter hibernation. Other complementary explanations could be related to composition of snail diet whose preferences vary widely from one area to another [42]. Nevertheless, RF/DM ratio recorded low variations in respect to area of origin and metabolic status. The last Ice Age started about 2 million years ago and ended only about 11,000 years ago [43]. In addition, in meat animals muscle/fat ratio presents a moderate coefficient of heritability: h^2 = 0.4-0.6 [44,45]. Since land snails survived throughout all this period we assume that they developed an evolved mechanism of hibernation that allowed them to pass overwinter with a relatively constant variation of foot RF/DM ratio within the same snail variety, regardless of location.

4.Conclusions

The following conclusions derive from the analysis of phenotype and metabolical status potential to induce changes of nutritive value and aperture parameters within the same variety of Roman snail (*Helix pomatia* var. Banatica): (1) shell aperture area tends to be smaller under drier conditions; (2) low correlations existing between the aperture height and the foot weight (R_s < ±0.2) suggest the lack of scientific background for calibration of wild snails; (3) variations of weight induced to different populations by distance and metabolic status seem rather limited to changes of live weight in respect to different climatic conditions than to variations of the foot one; (4) the foot raw protein content is a more promising indicator of phenotypical variability within the same species of gastropods than the raw fat content; (5) ratio between raw fat and dry matter content keeps relatively constant during wintertime, irrespective of climatic conditions, suggesting that snails developed an evolved homeostatic mechanism of hibernation.

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