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Preliminary Study on the Production Quality of Edible Snail *Cornu aspersum aspersum* (synonym *Helix aspersa aspersa*) Receiving a Feed Mix Supplemented with Betaine Hydrochloride (Trimethylglycine)

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Feed betaine (trimethylglycine) has appeared on the feed market relatively recently and its role as a dietary supplement in animal production is still subject to research. In the metabolic processes of a living organism, it may become a choline substitute (EFSA Journal, 2013). For example, in the final stage of pig breeding, there was no effect of betaine addition on the average animal weight increments but in a statistically significant way the feed consumption per unit of increment decreased and the carcase fat index decreased. The addition of betaine to feed or potable water increases the digestibility of some nutrients and can improve the quality of the carcass (Eklund et al., 2005). This quality, as well as the meat yield, in the case of snails are susceptible to some anti-stress and herbal-mineral feed additives (Ligaszewski et al., 2014). Quite often, in the final stage of the production cycle there appears a large amount of lipid tissue in the form of a jelly-like substance around the internal organs of maturing snails, especially from the subspecies *Helix aspersa maxima*.

It is the reason for a poor assessment of the quality of the carcass of snails sold to the processing plants, which is complained about by some producers and foreign importers. This phenomenon is not found in snails collected from natural populations or snails from French farms.

The reason for this may be the poor balancing of domestic mixtures for snails or the cooler climate of central Europe in comparison with the western part of the continent. Therefore, there is a need for further research of new feed additives that enable obtaining better results in the production quality under conditions of domestic heliculture.

Materials and methods

The tests were carried out on a semi-production scale (Experiment I) and in the laboratory conditions (Experiment II).

Experiment I

The greenhouse breeding was carried out within the area of earth farms with an area of 10 m^2, sown with a mix of turnip and fodder cabbage. Standard, dry plant feed for snails (with 18.2% protein content), containing experimental additions of betaine hydrochloride ($C_5H_{11}NO_2$.HCl) designed for general use feeders fed to snails on wooden pallets – feeders. When buying the above feed supplement Galvet offer was chosen. The enclosures, additionally equipped with a daily water srinkiel system, were supplied in the middle of May with the hatch of a small grey snail (*Cornu aspersum aspersum synonym Helix aspersa aspersa* The snail breeding took place based on own breeding population in the premises of the experimental farm of the Institute of Animal Production (PIB) in Balice.

The research was carried on a semi-production scale in a system of two experimental enclosures and two control ones, according to the following design:

Experimental enclosure 1 density of hatching snails: 300 pieces m^{-2} betaine hydrochloride content: 2.5 g kg^{-1} feed	**Experimental enclosure 2** density of snails hatching: 300 pieces m^{-2} betaine hydrochloride content: 5 g kg^{-1} feed
Control enclosure 1 density of hatching snails: 300 pieces m^{-2} feed without betaine hydrochloride content	**Additional control enclosure 2** (farther from the rest of the experimental block) density of hatching snails: 300 pieces m^{-2} feed without betaine hydrochloride

The experiment was carried out until the snails achieved somatic and commercial maturity. In random samples of 120 mature individuals from each enclosure, the body parameters of snails [body mass (g), shell diameter (mm)] and their meat yield were compiled, which consists of the coefficient of condition (g cm-3) expressed as the body mass divided by the cube diameter of the shell, the weight of the carcass (g) and the ratio of the carcass weight to the body weight of the snail expressed in % (Tab. 1). Collective tests of the experimental snails were then subjected to chemical analyses for the content of total protein and crude fat. The amino acid composition and profile of higher fatty acids were also examined.

Experiment II

In each of three breeding cuvettes placed in a laboratory room there were placed 50 pieces of 10-day-old hatch snails, so that their density corresponded to 300 pieces/m2. The following experimental design was used:

1) the control cuvette: the feed without the addition of betaine,
2) the experimental cuvette: the feed with the addition of 1.5 g of betaine in 1 kg of feed,
3) the experimental cuvette: the feed with the addition of 3.0 g of betaine in 1 kg of feed.

After the completion of the experiment, morphometric measurements of the body of all the mature snails were made and the meat yield parameters were examined in a wider range as compared to the greenhouse experiment (Tab. 2). This extension consisted in calculating the mass of the foot of the snail along with the collar (g) and the mass ratio to the body weight and the carcass of matured snails (%). The following chemical analysis methods were used:

- crude protein content marking:
  SOP M.007 in 2 of 21.02.2008 (AOAC, 2005);
- determination of free fat content: SOP M.013a PN-ISO 1444:2000 in 1 of 28.03.2011 (PN ISO 1442, 2000);
- the profile of higher fatty acids:
- P.015 in 1 of 22.05.2003. Gas chromatography method (ISO 12966-2, 2011);
- amino acid content marking in acid hydrolysates of feed and biological material.
- SOP M.004 in 7 of 16.03.2012, SOPM.005 in 7 of 16.03.2016 and SOP M.006 in 5 of 21.02.2008.

The statistically obtained results were characterised and compared using the Duncan Test and ANOVA, available in the statistical computer software of CSS Statistica (StatSoft Krakow).

Results

Body parameters and meat yield

In the semi-production breeding, mature snails which received fodder containing feed with betaine at a rate of 5.0 g kg^{-1} and 2.5 g kg^{-1}, did not reveal the statistically significant difference in body weight and the average shell size as compared to snails fed without this additive. However, the mass of shells and the condition coefficients were statistically significantly lower ($P < 0.01$) as compared to the control variant (Tab. 1).

However, in the next experiment conducted in the laboratory conditions, in which betaine content in the feed was reduced to 3.0 g kg^{-1} and 1.5 g kg^{-1} and the methods of meat yield evaluation were more precise by increasing the number of analysed parameters of this yield, a statistically beneficial effect of feeding snails with the feed containing this supplement was confirmed. It was found that with the addition of betaine in the feed in the amount of 3.0 g kg^{-1}, the average body weight and carcass mass of the snails was statistically significantly ($P < 0.05$) higher than in the control variant snails. For both variants of the feed with the addition of betaine, a statistically highly significant ($P < 0.01$) improvement in the body condition and greater leg mass as well as the ratio of leg weight to body weight and carcass weight were found as compared to the control feeding variant snails. Both in the production experiment as well as the laboratory tests, there were no statistically significant differences between the experimental variants and control variant for the diameter of snail shells and the ratio of their carcass weight to the total body weight.

Table 1. Snail body parameters and meat yield in an experiment performed in earth greenhouse enclosures (Experiment I)

Parameters	Betaine content in feed (g kg^{-1})		
	2,5	5,0	Control I
Body parameters			
Body weight (g)	9,19	9,17	9,53
Shell diameter (mm)	26,8	27,1	27,0
Shell weight (g)	**1,43^{A}**	**1,47^{A}**	**1,55^{B}**
Meat yield parameters			
Body condition index (g cm^{-3})	**0,47^{A}**	**0,46^{A}**	**0,48^{B}**
Carcass weight (g)	7,74	7,72	8,02
Carcass weight to body weight of mature snails (%)	84,0	84,2	84,4

a, b, c — significant differences ($P<0.05$) between treatments.
A, B, C – highly significant differences ($P<0.01$).

Table 1. Snail body parameters and meat yield in an experiment performed in earth greenhouse enclosures (Experiment II)

Parameters	Betaine content in feed (g kg^{-1})		
	1,5	3,0	Control I
Body parameters			
Body weight (g)	**10,4**	**10,66^{a}**	**9,98^{b}**
Shell diameter (mm)	25,9	26,0	25,9
Shell weight (g)	1,40	1,39	1,35
Meat yield parameters			
Body condition index (g cm^{-3}) (body weight : shell diameter $^{-3}$	**0,60^{A}**	**0,61^{A}**	**0,57^{B}**
Carcass weight (g)	9,0	**9,27^{a}**	**8,63^{b}**
Food weight (g)	**3,85^{A}**	**3,95^{A}**	**3,41^{B}**
Carcass weight to body weight of mature snails (%)	86,6	86,9	86,4
Foot weight to body weight of mature snails (%)	**37,2^{A}**	**37,2A**	**34,3B**
Foot weight to carcass weight of mature snails	**43,0^{A}**	**43,1A**	**40,2B**

a, b, c — significant differences (P<0.05) between treatments.
A, B, C – highly significant differences (P<0.01).

The results of basic chemical analyses of snail carcass

The survey tests carried out on collective samples of snail carcasses from the experience conducted on a semi-production scale showed that as the content of betaine increased, a small increase in the protein content in the carcasses occurred and, at the same time, the content of crude fat slightly decreased (Tab. 3). The analysis of profiles of higher fat fatty acids (WKT) showed a tendency to increase the PUFA-6/PUFA-3 ratio as the addition of betaine to feed increased. In the amino acid profiles a slight increase was observed in the share of glycine (Gly) and glutamic acid (Glu) as the proportion of betaine in the feed in the mixture increases (Tab. 4).

Discussion of the results

Gugołek et al. (2011) on the basis of the studies on mink feed proved that the implementation of betaine in the amount of 2-4 g/kg of feed increased the use of energy and digestibility of nutrients, especially protein while a higher dose (4 g kg^{-1}) improvement in digestibility (except protein) was lower than at 2 g kg^{-1}. The excessive use of betaine content may therefore bring negative or ambiguousproduction effects (Sales, 2011). This is due to the fact that excessive doses of betaine cause an increase in the concentration of LDL cholesterol in blood or fat tissue in animals and humans (Martins et al., 2010; EFSA Journal, 2011).

Therefore, for piglets/pigs and poultry, 2 g of betaine/1 kg of feed, 2-2.5 g/kg of feed for dairy cows and 1.5 g per person per day for humans was considered as the optimal dose (EFSA Journal report, 2013).

Table 3. Protein and crude fat content, PUFA-6/PUFA-3 ratio in carcasses of Cornu aspersum aspersum snails

Betaine content in feed (g kg)	*Protein* (g kg^{-1})	*Crude fat* (%)	PUFA-6 / PUFA-3
2,5	125	1,5	5,28
5,0	124	1,6	5,25
Control I	123	1,7	5,18
*Control II**	123	2,0	4,99

**Control II – commercial enclosure away from the blocked layout of experimental enclosures.*

Table 4. Amino acid profiles of protein in carcasses of snails from greenhouse enclosures. Collective samples

Betaine content in feed (g kg)	*Percentage of amino acids in profile*								
	1. Gly	2. Glu	3. Asp	4. Tre	5. Ser	6. Pro	7. Ala	8. Val	9. Ile
5,0	**7,2**	**13,0**	11,2	5,2	5,9	4,7	5,3	5,2	4,4
2,5	**6,9**	**12,8**	11,4	5,2	6,0	4,5	5,3	5,3	4,4
Control I	**6,5**	**12,8**	11,5	5,2	6,0	4,3	5,3	5,4	4,5
*Control II**	**6,1**	**12,5**	11,4	5,2	6,0	4,6	4,9	5,1	4,3

contd. Table 4. Amino acid profiles of protein in carcasses of snails from greenhouse enclosures. Collective samples

Betaine content in feed (g kg)	*Percentage of amino acids in rofile*								
	10. Leu	11. Tyr	12. Fen	13. His	14. Lis	15. Arg	16. Cys	17. Met	18. Trp
5,0	**6,1**	**3,7**	14,3	2,0	6,7	7,3	1,6	1,6	1,7
2,5	**6,2**	**3,8**	4,4	2,0	6,9	7,0	1,4	1,6	1,7
Control I	**6,3**	**3,9**	4,6	2,0	6,8	6,8	1,7	1,6	1,7
Control II	**6,2**	**4,4**	4,0	1,9	7,7	7,7	1,4	1,4	1,5

** Control II – commercial enclosure away from the blocked layout of experimental enclosures*

In the context of the above reports, betaine additives in the amount of 2.5-5.0 g kg^{-1} of feed could be too high for snails bred on the semi-production scale and, therefore, its application did not bring the expected results with respect to improving the quality of production. In the cuvette breeding, however, in laboratory conditions, a dose of 1.5 g kg^{-1} of feed had a statistically significant effect on the improvement of meat yield parameters, and the use of 3 g of betaine kg^{-1} of feed also increased the weight of mature snails. As opposite to the conditions of cuvette experimental rearing, snails in semi-production conditions were kept in earth greenhouse enclosures sown with fodder vegetation, which was

an additional source of food that could affect the food preferences of snails and, thus, the result of the research. An open issue therefore remains the palatability of snail feed containing higher feed betaine additives ranging from 2.5 g to 5.0 g kg^{-1}. Nonetheless, betaine additives in the above amount contributed to a slight increase in the share of protein and a lower content of crude fat in the carcass and also the PUFA-6/PUFA-3 ratio slightly increased. The increase in the addition of betaine to feed was also associated with a slightly higher share of glycine and glutamic acid (glutamine) in the amino acid profiles of the carcass protein. Glycine is one of the basic components of the collagen contained in an amount of about 2.0% in the smooth muscle of the leg (foot) of snails (Ligaszewski et al., 2005), but its increased share in the amino acid profile could also be directly related to the chemical structure of betaine consumed with fodder, which is a derivative of glycine.

Summary

In the semi-production conditions of earth enclosures sown with fodder vegetation, betaine admixtures in the amount of 2.5-5.0 g kg^{-1} of feed proved to be too high and counter-productive, without bringing the expected effects of improving the quality of production. In the closed laboratory conditions, however, without access to other sources of food, such as plants and soil, the dose of betaine at a rate of 1.5 g kg^{-1} of feed had a statistically significant effect ($P < 0.05$), and most often highly significant effect ($P < 0.01$) on the improvement of meat yield parameters. The application of 3 g betaine kg^{-1} feed additionally increased the statistically significant ($P < 0.01$) increase in the weight of mature snails. The addition of betaine in the amount of 1.5 g kg^{-1} and 3.0 g kg^{-1} resulted in a certain increase in the protein content in the carcass and a lower content of crude fat as well as in the PUFA-6/PUFA-3 ratio. The effect was also an increase in the share of glycine and glutamic acid (glutamine) in the amino acid composition of the carcass protein.

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PRELIMINARY STUDY ON THE PRODUCTION QUALITY OF EDIBLE SNAIL *CORNU ASPERSUM ASPERSUM* (SYNONYM *HELIX ASPERSA ASPERSA*) RECEIVING A FEED MIX SUPPLEMENTED WITH BETAINE HYDROCHLORIDE (TRIMETHYLGLYCINE)

Summary

Two laboratory and semi-production feeding experiments were performed to determine changes in some production traits of edible snail *Cornu aspersum aspersum* (synonym *Helix aspersa aspersa*) during ontogenic development. Snails were fed a standard dry loose-mix diet for snails, supplemented with feed-grade betaine hydrochloride (trimethylglycine). The betaine hydrochloride was supplemented at 1.5 g kg^{-1} and 3.0 g kg^{-1} feed in the laboratory experiment, and at 2.5 g kg^{-1} and 5.0 g kg^{-1} under semi-production conditions The control diets fed to snails in both experiments did not contain this supplement. It was found that under semi-production conditions of earth enclosures sown with fodder vegetation, betaine supplements of 2.5 and 5.0 g kg^{-1} feed proved too high and did not yield the expected results of higher body weight or improved meat yield of the commercial snails. Nonetheless, the additive supplemented at the above levels contributed to a slight increase in carcass protein content, a higher PUFA-6/PUFA-3 ratio in the profile of higher fatty acids, and a marked increase in glycine percentage in the amino acid profile of protein, while reducing the crude fat content compared to the results of feeding the control diet. For snails confined to laboratory rearing, where no other feed sources such as vegetation and soil were available, a betaine dose as low as 1.5 g kg^{-1} feed caused a significant ($P<0.05$), and most often a highly significant ($P<0.01$) improvement in meat yield parameters. In addition, the use of 3 g betaine hydrochloride kg^{-1} feed caused a significant ($P<0.05$) increase in the body weight of mature snails.

Key words: *Cornu aspersum*, snail feeding, production quality, milk composition, milk yield