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EFFECT OF AGED GARLIC EXTRACT AND ALLICIN ADMINISTRATION TO SOWS DURING PREGNANCY AND LACTATION ON BODY WEIGHT GAIN AND GASTROINTESTINAL TRACT DEVELOPMENT OF PIGLETS. PART I.

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Abstract

The aim of this study was to investigate the influence of perinatal administration of aged garlic extract (AGE) and allicin to pregnant sows on body weight gain and gastrointestinal tract development in their piglets. The animals were kept under standard rearing conditions and fed well balanced diet *ad libitum*. The piglets were obtained from 18 sows and divided into 3 equal experimental groups. Moreover, the experimental animals were divided additionally into 8 age-differentiated subgroups, namely non-suckling newborn piglets and piglets 1, 3, 7, 14, 28, 35, and 56 d of age. Starting from the 91st d of pregnancy up to piglets' weaning on the 28th d of their life, the sows were daily treated *per os* with AGE or allicin, whereas the control group received the vehiculum. Daily body weight gain was estimated. To determine the weight of internal organs and length of the small intestine, the piglets were sacrificed according to the experimental design and the gastrointestinal tract was isolated. The positive influence of AGE and allicin administered to pregnant and lactating sows on body weight gain and gastrointestinal tract development of piglets was demonstrated. It seems that garlic supplements may be considered as an attractive alternative for antibiotics that are widely used in pigs' nutrition.

Key words: piglets, aged garlic extract, allicin, weight gain, gastrointestinal tract development.

The *Allium* genus of vegetables includes garlic, onions, leeks, scallions, chives and shallots. The medical uses of garlic (*Allium sativum*) have a long history. Interest in the potential benefits of garlic has origins in antiquity and is one of the earliest documented examples of plants used for maintenance of health and treatment of disease (11). Hundreds of chemical substances are present in fresh, dried or extracts of garlic. Significant synergy or antagonism of the garlic substances influencing human and animal physiology may exist and vary with an individual stage of development, pathology, dosage regimen and possible drug, food or metabolite interactions. The chemicals present in a garlic product are dependent on potential substrate activity of the enzyme allicinase that is sequestered within garlic cell vacuoles, temperature, and duration of drying, use of polar and/or non-polar extraction solvents as well as conditions and period of maceration before final extraction. Moreover, crushing of the garlic cells allows alliinase to interact with alliin and form allicin (12). Among many preparation processes of garlic products the most common are freeze-drying, low temperature drying, distillation, maceration in oil, hydroalcoholic short extraction and hydroalcoholic long maceration. The freeze-drying of fresh garlic is a method of flash evaporation at low temperature in a partial vacuum and results in virtually no changes in chemical compositions. The resulting product is usually used for culinary purposes. The low temperature drying process involves drying fresh cloves at <50^{o}C for 3-4 d and allicin formation that is converted to allyl sulphides. The final product has many of the attributes of the fresh garlic clove that include γ-glutamyl cysteine, the precursor to allin and S-allylcysteine (SAC). Steam distilled garlic contains principally allyl sulphides. During oil maceration process chopped garlic is homogenized and slowly extracted in vegetable oil. Such products contain vinyldithiins, allyl sulphides and ajoene. Whereas the stability of the constituents of fresh or dried garlic extracted with a hydroalcoholic solution for short maceration is questionable, the major ingredient of the hydroalcoholic long maceration of sliced garlic is S-allylcysteine (12). However, the aged garlic extract (AGE) is achieved after long extraction from 6 up to 20 months. During this aging process, the

odorous, harsh and irritating compounds in garlic are converted into stable and safe sulphur compounds such as S-allylcysteine and S-allyl mercaptocysteine (SAMC). Moreover, next to water soluble SAC and SAMC, AGE consists of mainly small amount of various oil-soluble compounds (1, 7, 16).

Considering studies that confirmed wide range of positive effects of garlic and its compounds or their metabolites in humans and animals it was decided to investigate the effect of aged garlic extract and allicin administration in pregnant and lactating sows on body weight gain and gastrointestinal tract development in their piglets. AGE and allicin were selected for this study to investigate effects of water and oil soluble compounds of garlic, respectively, on developmental processes of piglets. Whereas allicin is known to have the most effective antimicrobial activity of garlic compounds, AGE is reported as the most suitable form among garlic preparations for the long-term use (6, 13). Moreover, our earlier studies with AGE and allicin showed beneficial influence of these substances on non-specific and specific defense mechanisms of sows and piglets. These positive effects may be coupled with the improvement of animal growth rate and gastrointestinal tract development (3, 14).

Material and Methods

Experimental design and sampling procedure. The experimental procedures used throughout this study are in compliance with the guidelines for the "Care and Use of Animals" as published by the American Journal of Physiology, and were approved by the Local Ethic Committee on Animal Experimentation of the Agricultural University of Lublin, Poland.

The experiment was performed on piglets born by sows of the Large Polish White breed. The animals were kept under standard rearing conditions with free access to fresh water and fed well balanced diet. The piglets obtained from 18 sows were divided into three equal experimental groups. The experimental animals were divided additionally into 8 age-differentiated subgroups (each subgroup n = 6), namely non-suckling newborn piglets and piglets 1, 3, 7, 14, 28, 35, and 56 d of age. Starting from the 91st d of pregnancy up to piglets' weaning on 28th d of their life, the sows were daily treated *per os* during the morning meal with AGE (n = 6) at the dosage of 10 ml/100 kg b.w. or allicin (n = 6) at the dosage of 1.6 mg/ 100 kg b.w., whereas the controls (n = 6) received 10 ml/100 kg b.w. of a vehiculum. The piglets from AGE- or allicin-treated sows were assigned to AGE and ALL groups, respectively. Whereas AGE was prepared as a result of 12-months long hydroalcoholic maceration of sliced garlic, according to the instructions presented by Staba (12), the allicin was administered in the form of commercial capsular preparations (Alliomax, Herbapol Lublin S. A., Poland). All the piglets were weighed every day to determine body weight gain. The stomach, liver, pancreas and small intestine were isolated from newborn piglets as well as from piglets at the age of 1, 3, 7, 14, 28, 35, and 56 d of life. To avoid sex-conditioned differences of the gastrointestinal tract, the sacrificed piglets within age-differentiated subgroups were sex-matched. Moreover, just after the gastrointestinal tract isolation, the weight of empty stomach, liver and pancreas was taken. In the next step, the mesentery from the intestine was removed gently and the length of the duodenum and total small intestine was measured.

Statistical analysis. Statistical analysis was performed using Statistica software (version 6.0). All data are presented as means ± SEM. All investigated parameters in all groups were found to be normally distributed in accordance with Kolomogorov-Smirnov test and have equal variance. Differences between AGE or ALL groups *versus* control group were tested for statistical significance with the use of Student's *t*-test. Differences showing $P \leq 0.05$ were considered significant.

Results

The obtained results showed significant increase in body weight values in newborn piglets in both experimental groups when compared to the controls. Furthermore, the body weight was significantly increased in piglets from AGE and ALL groups at the age of 1, 3, 7, 14, 28, 35, and 56 d of life (Table 1).

Except for the newborn piglets, the weight of the liver of piglets from AGE group reached significantly higher values in all other subgroups. Maternal administration of allicin induced the higher weight of the liver in piglets at the age of 1, 3, 14, and 56 d of life when compared to the control group (Table 2).

The piglets from AGE group were characterized by significantly higher weight of the pancreas when examined in newborn ones and at the age of 14, 28, 35, and 56 d of life. Moreover, except for the 1 week old piglets allicin administration increased this parameter in all other subgroups when compared to the controls (Table 3).

The weights of the stomach in piglets from all groups are presented in Table 4. Aged garlic extract administration to sows induced significantly higher weight of the stomach in piglets at the age of 7, 14, 28, and 56 d, whereas allicin administration increased this parameter in the newborn piglets and at the age of 3, 14, 28, and 56 d of life.

The duodenum of piglets from AGE group was significantly longer in the newborn piglets and at the age of 3, 7, 14, 35, and 56 d. Moreover, the length of the small intestine was increased in 1, 7, 14, 28, 35, and 56 d old piglets from this group. The piglets from ALL group reached higher values of the duodenum length just after the birth and at the age of 35 and 56 d of life when compared to the control group. The length of the small intestine was increased in the animals from this group at the age of 1, 14, and 56 d (Table 5).

The ratio of liver to body weight in 1 week old piglets from AGE group was significantly increased when compared to the control group. Similar results were observed in 5 and 8 weeks old piglets from this group when the ratio of pancreas to body weight was analysed. Moreover, allicin administration enhanced this parameter in 8 week old animals (Table 6).

Table 1
Body weight (g) of newborn and 1, 3, 7, 14, 28, 35, and 56 d of age piglets

Age (d)	Control group	AGE group	ALL group
Newborn	1 161 (±73)	1 378 (±35)*	1 522 (±55)*
1	1 249 (±88)	1 567 (±39)*	1 638 (±88)*
3	1 540 (±104)	2 022 (±52)*	2 035 (±93)*
7	2 435 (±185)	3 032 (±66)*	3 042 (±162)*
14	3 722 (±273)	4 934 (±143)*	4 968 (±347)*
28	6 287 (±539)	8 855 (±236)*	8 792 (±666)*
35	7 640 (±517)	9 512 (±376)*	10 083 (±378)*
56	13 928 (±838)	18 288 (±787)*	18 775 (±785)*

* P ≤ 0.01 *versus* control group.

Table 2
Weight of the liver (g) of newborn and 1, 3, 7, 14, 28, 35, and 56 d of age piglets

Age (d)	Control group	AGE group	ALL group
Newborn	31.4 (±2.4)	35.8 (±3.5)	31.1 (±2.4)
1	39.2 (±3.2)	49.5 (±2.9)*	53.5 (±3.3)*
3	50 (±0.6)	72.2 (±1.1)**	78.8 (±3.5)**
7	76.2 (±2.7)	116.0 (±4.2)**	80.2 (±3.2)
14	111.6 (±6.1)	145.8 (±4.2)**	132 (±6.1)*
28	170.8 (±14.0)	232.4 (±5.8)*	171.3 (±10.8)
35	239.6 (±19.5)	294.5 (±9.9)*	242.2 (±12.5)
56	492.2 (±31.0)	662.0 (±25.5)**	598.5 (±14.2)*

* P ≤ 0.05; ** P ≤ 0.01 *versus* control group.

Table 3
Weight of the pancreas (g) of newborn and 1, 3, 7, 14, 28, 35, and 56 d of age piglets

Age (d)	Control group	AGE group	ALL group
Newborn	0.83 (±0.08)	1.30 (±0.06)**	1.58 (±0.11)**
1	2.53 (±0.09)	2.46 (±0.11)	3.40 (±0.11)**
3	3.0 (±0.19)	3.35 (±0.13)	4.21 (±0.11)**
7	5.30 (±0.30)	5.93 (±0.20)	5.15 (±0.15)
14	5.90 (±0.41)	8.60 (±0.66)**	7.30 (±0.29)*
28	10.36 (±0.96)	15.68 (±0.65)**	13.78 (±0.37)*
35	14.10 (±1.22)	21.73 (±1.06)**	18.80 (±1.12)*
56 d of life	26.46 (±2.32)	46.53 (±1.97)**	53.50 (±2.68)**

* P ≤ 0.05; ** P ≤ 0.01 *versus* control group.

Table 4
Weight of the stomach (g) of newborn and 1, 3, 7, 14, 28, 35, and 56 d of age piglets

Age (d)	Control group	AGE-treated group	ALL-treated group
Newborn	4.0 (±0.43)	5.31 (±0.55)	6.7 (±0.46)**
1	7.06 (±0.47)	8.16 (±0.21)	8.86 (±0.55)
3	9.3 (±0.55)	10.65 (±0.27)	12.56 (±1.07)*
7	13.36 (±1.36)	18.50 (±0.94)**	14.90 (±0.50)
14	19.03 (±1.15)	22.40 (±0.61)*	26.06 (±0.99)**
28	35.80 (±2.03)	57.15 (±2.45)**	42.65 (±2.16)*
35	55.96 (±1.76)	63.90 (±2.12)	58.70 (±2.32)
56	166.10 (±5.71)	212.40 (±18.00)*	212.00 (±12.35)**

* $P \leq 0.05$; ** $P \leq 0.01$ *versus* control group.

Table 5
Length of the duodenum and small intestine (cm) of newborn and 1, 3, 7, 14, 28, 35, and 56 d of age piglets, measured after removing the mesentery

Age (d)	Control group		AGE group		ALL group	
	Duodenum	Small intestine	Duodenum	Small intestine	Duodenum	Small intestine
Newborn	13.2 (±0.8)	292 (±34)	16.8 (±0.7)*	315 (±6)	17.3 (±0.7)**	328 (±17)
1	15.3 (±0.9)	384 (±18)	19.6 (±1.8)	475 (±29)*	17.5 (±0.8)	460 (±13)**
3	19.2 (±0.8)	478 (±17)	23.5 (±1.6)*	498 (±21)	18.2 (±0.7)	498 (±14)
7	21.5 (±0.9)	520 (±18)	28.2 (±0.8)**	594 (±18)**	21.6 (±1.6)	521 (±15)
14	25.5 (±1.5)	628 (±21)	31.2 (±1.6)*	693 (±13)*	27.3 (±1.7)	717 (±34)*
28	27.0 (±1.9)	803 (±13)	33.2 (±0.8)	1032 (±40)**	31.2 (±2.2)	893 (±70)
35	29.0 (±1.4)	903 (±31)	37.5 (±2.0)**	1258 (±61)**	34.5 (±1.8)*	1003 (±47)
56	34.8 (±1.6)	1070 (±46)	41.2 (±1.4)**	1321 (±43)**	41.5 (±2.2)*	1213 (±40)*

* $P \leq 0.05$; ** $P \leq 0.01$ *versus* control group.

Table 6

Ratio of the liver (L), pancreas (P) and stomach (S) to body weight (BW) in newborn and 1, 3, 7, 14, 28, 35, and 56 d of age piglets

Age (d)	Control group			AGE group			ALL group		
	L:BW	P:BW	S:BW	L:BW	P:BW	S:BW	L:BW	P:BW	S:BW
Newborn	0.031 (±0.001)	0.0008 (±0.00007)	0.0039 (±0.00010)	0.026 (±0.001)	0.00097 (±0.00007)	0.0039 (±0.00053)	0.021 (±0.002)**	0.0010 (±0.00009)	0.0045 (±0.00043)
1	0.034 (±0.001)	0.0022 (±0.00010)	0.0061 (±0.00023)	0.034 (±0.001)	0.0017 (±0.00009)**	0.0055 (±0.00024)	0.033 (±0.0009)	0.0021 (±0.00007)	0.0054 (±0.00018)*
3	0.039 (±0.001)	0.0020 (±0.00011)	0.0063 (±0.00041)	0.037 (±0.0004)	0.0017 (±0.00006)	0.0055 (±0.00008)	0.039 (±0.0009)	0.0021 (±0.00006)	0.0061 (±0.00035)
7	0.032 (±0.0005)	0.0022 (±0.00008)	0.0056 (±0.00070)	0.039 (±0.0019)**	0.0020 (±0.00012)	0.0063 (±0.00040)	0.028 (±0.0012)*	0.0018 (±0.00012)*	0.0053 (±0.00050)
14	0.033 (±0.0003)	0.0017 (±0.00006)	0.0056 (±0.00036)	0.031 (±0.0005)*	0.0018 (±0.00017)	0.0047 (±0.00030)	0.028 (±0.0006)**	0.0015 (±0.00003)	0.0055 (±0.00014)
28	0.028 (±0.0008)	0.0017 (±0.00012)	0.0061 (±0.00065)	0.028 (±0.0010)	0.0018 (±0.00007)	0.0069 (±0.00033)	0.021 (±0.0017)**	0.0017 (±0.00010)	0.0052 (±0.00040)
35	0.029 (±0.0007)	0.0017 (±0.00013)	0.0071 (±0.00073)	0.030 (±0.0012)	0.0022 (±0.00012)*	0.0065 (±0.00020)	0.025 (±0.0010)**	0.0019 (±0.00006)	0.0060 (±0.00009)
56	0.035 (±0.0015)	0.0019 (±0.00016)	0.012 (±0.00052)	0.036 (±0.0012)	0.0026 (±0.00013)**	0.012 (±0.0010)	0.032 (±0.0006)	0.0029 (±0.00015)**	0.011 (±0.00074)

* $P \leq 0.05$; ** $P \leq 0.01$ *versus* control group.

Discussion

The earliest known references indicate that garlic was a part of the daily diet of Egyptians, Babylonians, Phoenicians, Vikings, Chinese, Greeks, Romans and Hindus. It was fed commonly to the working class involved in heavy labour, as in the building of the pyramids (9). Moreover, garlic served as remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging and many other ailments (1). It was believed that garlic increased strength in humans, enabling harder work and higher productivity (9). The *Codex Ebers*, authoritative medical text, is one of the earliest sources indicating prescription of garlic for the treatment of malignancies called "abnormal growths". It was applied for abscesses, circulatory ailments, general malaise, and infestations with parasites (11). Other investigation showed that oil-soluble allicin has immunostimulatory, antimicrobial, and anti-tumour effects (1, 2, 10).

Even though beneficial effects of garlic in humans are widely investigated, the effects of garlic supplements administration in farm animals are poorly documented, especially when perinatal period is considered. Our investigations showed that oral administration of aged garlic extract and allicin to pregnant sows and during 28 d of lactation induced improvement of body weight of the piglets. Next to the higher body weight values obtained after AGE or allicin administration, the weight of the liver and pancreas were higher as well. Furthermore, the weight of the stomach and length of the duodenum and small intestine in piglets from groups treated with AGE and allicin were increased. In addition, similar values of ratios of the internal organs to body weight in both experimental groups compared with the control group indicate parallel growth rate of the whole body of piglets. These results are in contrasts to observations of Horton *et al*. (4) who revealed no effect of fresh garlic administration on body weight gain in piglets; however, this study was conducted on weaned animals. On the other hand, broiler chickens were characterized by significantly increased body weight gain, when garlic was added to the feed during the first 21 d of life (5). These results suggest that garlic compounds induce positive effects on the growth and productivity of animals, when administered at early stages of their development. Moreover, prenatal susceptibility of organisms to modulation of their development may be higher than during postnatal life. Turner *et al*. (15) demonstrated the positive influence of garlic administration on the growth and feed efficiency in weaned piglets in one of four studies performed. Thus, perinatal administration of garlic preparations like AGE and allicin seems to be very effective for the stimulation of developmental processes of piglets. Considering our results, it is suggested that increased body weight gain in piglets from AGE- or allicin-treated sows may be induced by improvement of gastrointestinal tract development and functions. Due to increased body weight values at the birth and during the postnatal life of piglets, AGE and allicin may be considered effective factors for the stimulation of organism development. These observations seem to be very important, considering current conditions of swine production, directed for optimal preparation of piglets to early weaning. Regarding the obtained results and immunostimulatory effects on non-specific and specific defense mechanisms of piglets observed in our earlier studies, garlic supplements may be recognized as an attractive alternative for antibiotics that are widely used in pigs' nutrition and are connected with negative consequences relating to humans (14). This hypothesis is supported by results obtained in other study, where garlic supplement was administered to piglets from 2 to 6 weeks of life. Next to the higher growth rate, *in vitro* investigation showed antibacterial effects of allicin on *Escherichia coli* isolated from the gastrointestinal tract of piglets (8).

In conclusion, this study showed positive influence of AGE and allicin as garlic products on body systemic development of piglets and should be further investigated in relation to intestine microstructure and digestive enzymes activity to explore mechanisms responsible for these beneficial effects.

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