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RESEARCH ARTICLE

Effect of Nettle Extract on Metabolic Processes in Piglets During Weaning

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Abstract

The article deals with the use of a plant extract to improve metabolic processes and the adaptive capacity of piglets during the weaning period. The study focused on the effects of a 40% ethanolic extract of nettle *(Urtica dioica* L.) on hematological and biochemical parameters in the blood of piglets. The piglets were divided into two groups - control and experimental with 9 animals in each group. From 14 days of age until weaning, the experimental animals were fed with the nettle extract at a rate of 6 mg/kg body weight for 22 days. Blood samples were collected at 14, 36, and 42 days of piglets' age. The study found that the nettle extract improved the blood respiratory function and positively influenced protein and energy metabolism in piglets. The nettle extract led to increased hemoglobin concentration, erythrocytes and leukocyte count, total protein, transaminases activity, glucose concentration, alkaline phosphatase and creatine kinase activity. The piglets fed with nettle extract showed a 10% higher live weight and average daily gain at 42 days of age, and a 12% higher safety rate compared to piglets fed with a standard diet. The results indicate that adding 40% nettle extract to the standard piglets' diet can stimulate metabolic and adaptive processes, as well as improve productive performance during the critical period after weaning.

Keywords: *Urtica dioica* L. extract, biochemical and hematological parameters, weaning stress, piglets

INTRODUCTION

Numerous studies have shown that weaning is the most challenging period for pigs, affecting their welfare and productivity significantly [1-3]. During this time, piglets are exposed to several factors, including psychosocial (separation from the mother and movement to new groups), immunological, infectious, metabolic, and nutritional stress. These factors often result in diarrhea and intestinal damage, which can negatively impact the survival rate of young animals. The post-weaning mortality rate can range from 6-10% and can sometimes go up to 20%. To address this, animal nutritionists have been working to optimize feed composition to meet the needs of newly weaned pigs and have been exploring various nutritional factors and housing conditions to promote piglet health [4-6].

Herbs and essential oils are used in pig nutrition due to their antibacterial, anti-inflammatory, antioxidant, and antiparasitic properties. The use of herbs in pig nutrition has shown many positive results, such as improving the taste of feed, increasing appetite, regulating digestion and metabolic processes, and lengthening intestinal villi, which improves the digestibility and digestion of the feed ration [7,8]. Researchers have also found that herbs decrease post-weaning diarrhea in piglets by reducing the number of Escherichia coli and ammonia emissions [9].

Common nettle *(Urtica dioica L.)* is a wild herbaceous perennial flowering plant that grows in Europe, Asia, North Africa, and North America. Nettle is known for its anti-inflammatory, anti-proliferative, antioxidant, analgesic, anti-infective, hypotensive, and anti-ulcer properties. It can reduce the intensity of hormonal changes in the body, act as a coagulant, and be used to make fertilizers and insecticides. The plant's multifaceted chemical composition, which includes carboxylic acids, amino acids, lipids and fatty acids, nitrogen-containing compounds, essential oils, steroids, lectins, lignans, coumarins, histamine, pigments, vitamins, tannins, flavonoids, trace elements,

and macronutrients, determines its high pharmacological properties [10,11]. Because of the vast array of natural biologically active compounds that make up nettle, and the large amounts of environmentally friendly raw materials available on almost all continents, it's possible to isolate these compounds and develop new drugs and food additives from nettle extract. These extracts can increase adaptive capacity, and correct metabolic disorders in animals and humans, making this research extremely relevant [12,13].

The study aimed to determine the effect of ethanol extract of common nettle on some biochemical, hematological, and productive parameters in piglets during weaning.

Material and Methods

Ethical Statement

The permission to perform the experiments was obtained from the Bioethics Committee of the Institute of Animal Biology NAAS of Lviv, Ukraine, approval No 77 of 20 December, 2021. All experiments were conducted according to the EU Directive 2010/63/EU for animal experiments.

Experimental Plant, Collection, Identification and Preparation

The common nettle *(Urtica dioica L.)* was harvested in the Skole district, located in the Carpathian highlands. The plant was identified as the common nettle *(Urtica dioica* L.) using the atlas-identifier of plants of Ukraine [14]. Nettle air parts were dried under normal conditions (in a dark place, temperature 20-25°C, relative humidity 30-60%). The dry material was then crushed to a particle size of 1.5 mm and placed in the extractor. The extraction process took eight days at a temperature of 20°C. We used a classical maceration with the ratio of raw material to 40% water-alcohol solution as an extractant (1:20, m/v). After extraction, the nettle extract was filtered and dehydrated using a rotary evaporator until it acquired a powdery form. For the experiment, the dry extract was used.

Experimental Animals and Design

The experiment was performed on 14-day-old piglets of a large white breed with a live weight from 5.18 to 5.48 kg. After farrowing two groups (control and experimental) were formed: 3 sows with piglets in each group. Each sow was kept in a separate cage with piglets (8-10 heads). On the 35th day after birth, piglets were weaned and each litter was kept in a separate weaner cage. Animals were fed a standard diet *ad libitum*, using a premix from Sano (Ferkengold Forte) for weaned piglets with free access to feed and water.

Piglets of the experimental group (Nettle) received a 40% extract of nettle in the dose of 6 mg/kg of body weight. The feed was mixed with a dry nettle extract and added to the feeders each morning for a group of animals in the cage from the 14th day after birth and to the weaning day (35th day of age). The extract feeding period lasted 22 days. At the same time, sows did not have access to the feed crib. The piglets of the control group (Control) were kept on a standard diet. The experiment lasted 30 days. Blood of piglets for the study (3 heads from each cage, 9 animals from the group) was taken before morning feeding from the anterior vena cava at 14, 36 (1 day after weaning), and 42 days of age (7 days after weaning). The survival and morbidity of piglets of both groups were monitored during the experiment. At the beginning and at the end of the experiment, the piglets were weighted.

Sampling Procedures

Piglets' blood and plasma were studied. 1% solution of heparin was used as an anticoagulant. The blood plasma was separated by centrifugation at 700 g for 15 min. Hematological parameters (number of erythrocytes and leukocytes in the Goriayev chamber) were determined in the blood and hemoglobin concentration was studied by hemoglobin-cyanide method [15].

Biochemical Analysis

The total protein in blood plasma was determined by the Lowry method [16], glucose concentration was measured by the glucose oxidase method [15], the activities of the creatine kinase (CK, EC 2.7.3.2), alkaline phosphatase (ALP, EC 1.11.1.7), alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1) - using the kits "Simko LTD". The absorbance values were measured on a spectrophotometer "Unico" 1205 (USA).

Statistical Analysis

The result among the groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. Statistical difference was considered significant at $P<0.05$. Results were expressed as mean ± Standard error of mean.

Results

In the animals of the experimental group, nettle extract caused a significant increase in hemoglobin concentration by 6% ($P<0.001$) and 12% ($P<0.001$) on days 1 and 7 after weaning from sows (*Fig. 1*).

During the experiment, it was observed that 36 and 42-day-old piglets in the experimental groups had 8% ($P<0.01$) and 7% ($P<0.01$) more erythrocytes than the control group, respectively. In addition, the number of leukocytes in the experimental group on the 1 day after weaning was found to be 5% higher than that of the control group ($P<0.001$) *(Fig. 2-A,B)*. At 42 days of age, both the

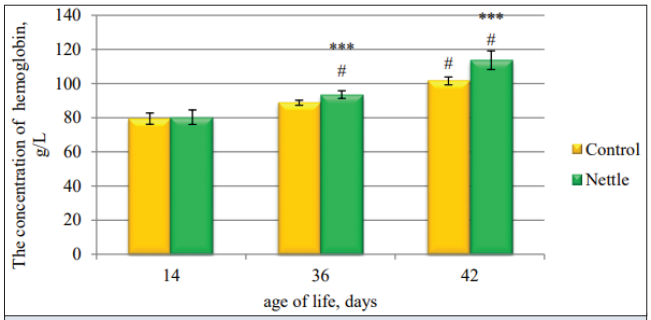

Fig 1. The concentration of hemoglobin in the blood of piglets (M ± SE, n = 9). * the differences are significant between the control and experimental groups of piglets (* P<0.05; ** P<0.01; *** P<0.001); # the differences are significant compared to 14-day-old animals (# P<0.05; ## P<0.01; ### P<0.001); Control - control group of piglets; Nettle - experimental piglets, which were fed additionally to the standard diet 40% extract of nettle *(Urtica dioica* L.) in the dose of 6 mg/kg of body weight

control and experimental groups showed a significant increase in the concentration of Hb (P<0.05) *(Fig. 1)* and the number of erythrocytes (P<0.01) *(Fig. 2-A)*. Similarly, the number of leukocytes increased significantly on days 36 and 42 of life in both groups compared to the beginning

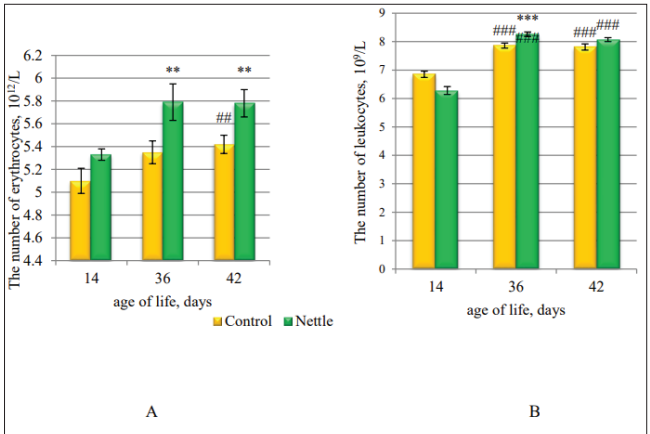

Fig 2. The number of erythrocytes (A) and of leukocytes (B) in the blood of piglets (M ± SE, n = 9). * the differences are significant between the control and experimental groups of piglets (* P<0.05; ** P<0.01; *** - P<0.001); # - the differences are significant compared to 14-day-old animals (# P<0.05; ## P<0.01; ### P<0.001)

of the experiment, with an average increase of 1.3 times (P<0.001) *(Fig. 2-B)*.

Our results have shown that at 36 and 42 days of age, the experimental group of piglets showed a significant increase in the content of total protein in their blood plasma by 15% (P<0.001) and 6% (P<0.001) respectively, compared to the control. It's worth noting that the total protein in the control group of piglets significantly decreased by 5% (P<0.01) on the first day after weaning, while the experimental piglets' concentration increased by 11% compared to the beginning of the study (P<0.001). By day 42, the total protein in the blood of animals significantly increased by 15% (P<0.001) in the control group and 23%

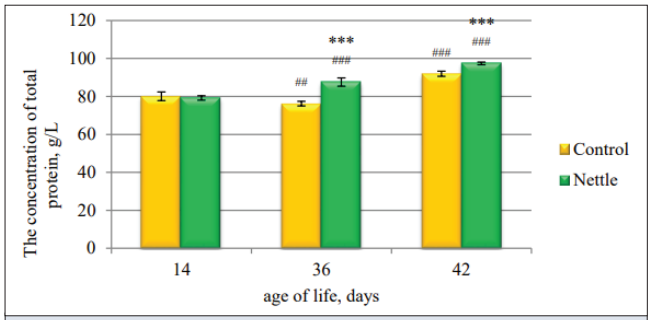

Fig 3. The concentration of total protein in the blood of piglets (M ± SE, n = 9). * the differences are significant between the control and experimental groups of piglets (* P<0.05; ** P<0.01; *** P <0.001); # the differences are significant compared to 14-day-old animals (# P<0.05; ## P <0.01; ### P <0.001)

(P<0.001) in the experimental group compared to day 14 of life *(Fig. 3)*.

In the experimental group of animals, an increase in the activity of AST by 25% (P<0.001) and 15% (P<0.001) and ALT by 2.1 (P<0.001) and 1.5 (P<0.01) times was observed within the normal physiological range, on the 1 and 7 days after weaning, respectively, as compared to the control group. Both enzymes showed an increase in their activity at the same age periods in the blood of experimental piglets by 2 (P<0.001) and 1.5 (P<0.001) times, compared to 14-day-old animals However, in control animals, the activity of these enzymes remained at the same level throughout the experimental period *(Fig. 4)*.

At 1 and 14 days after weaning, a significant increase of glucose in the blood plasma of the experimental animals compared to the control by 11% (P<0.01) and 10% (P<0.01), respectively, was found. Moreover, the decrease in the content of this metabolite was found in 36-day-old control and experimental piglets by 21% (P<0.05) and 12 % (P<0.05), respectively, compared to the beginning of the study, as shown in *Fig. 5*.

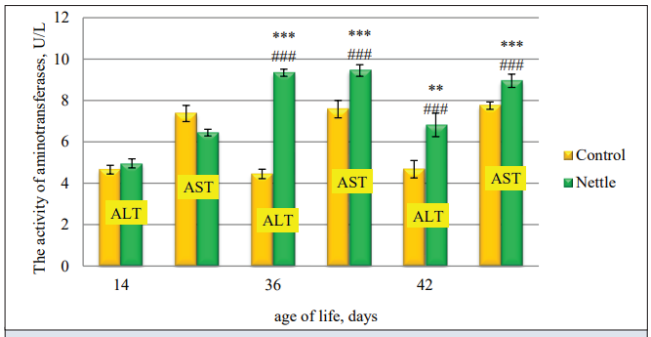

Fig 4. The activity of aminotransferases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) in the blood of piglets (M ± SE, n = 9). * the differences are significant between the control and experimental groups of piglets (* P<0.05; ** P<0.01; *** P<0.001); # the differences are significant compared to 14-day-old animals (# P<0.05; ## P<0.01; ### P<0.001)

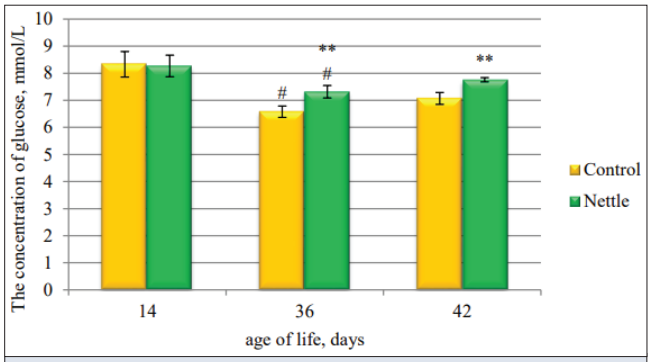

Fig 5. The concentration of glucose in the blood of piglets (M ± SE, n = 9). * the differences are significant between the control and experimental groups of piglets (* P<0.05; ** P<0.01; *** P<0.001); # the differences are significant compared to 14-day-old animals (# P<0.05; ## P<0.01; ### P<0.001)

In the study, it was found that the activity of ALP and CK increased significantly in the blood of piglets subjected to nettle treatment at 36 and 42 days of age compared to animals on a standard diet. The activity of ALP increased by 25% (P<0.001) on the first day after weaning and by 10% (P<0.01) on the seventh day in comparison with control group, while decreasing in both groups of piglets by three times compared to 14-day-old animals (P<0.001) *(Fig. 6-A)*. The activity of CK was also found to be significantly increased in 36-day-old experimental animals by 1.4 times (P<0.001) and in 42-day-old animals by 1.8 times (P<0.001) compared to the control group. Furthermore, the activity of CK increased significantly in the blood of both control and experimental piglets by 1.4 times (P<0.001) and 2 times (P<0.001) respectively on day 1 after weaning. However, on day 14 in control piglets, the activity of CK decreased by 1.4 times (P<0.001), whereas in experimental animals, it remained 1.3 times higher (P<0.001) *(Fig. 6-B)*.

The performance of piglets of both groups was determined for the experiment. At 14 days after birth, both groups had a live weight between 5.18-5.48 kg. On day 7 after

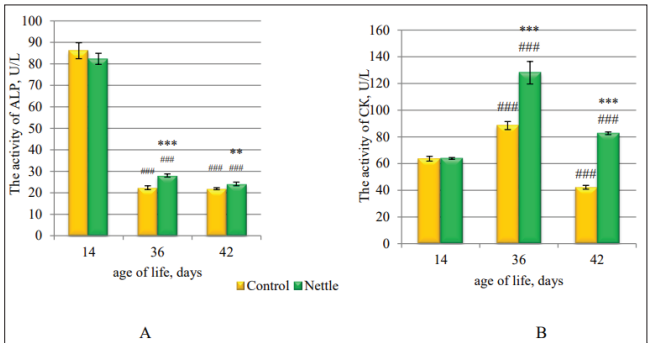

Fig 6. The activity of alkaline phosphatase (ALP (A)) and creatine kinase (CK (B)) in the blood of piglets (M ± SE, n = 9). * the differences are significant between the control and experimental groups of piglets (* P<0.05; ** P<0.01; *** P<0.001); # the differences are significant compared to 14-day-old animals (# P<0.05; ## P<0.01; ### P<0.001)

weaning (42 days of age), piglets in the control group had an average weight of 9.3 kg (with an average daily gain of 221 g), while piglets treated with nettle extract had an average weight of 10.25 kg (with an average daily gain of 244 g). Therefore, the addition of nettle extract to the piglets' diet led to a 10% increase in live weight and average daily weight gain compared to the standard diet. Moreover, the experimental group of piglets had a 12% higher survival rate than the control group.

Discussion

As already mentioned, weaning piglets from sows causes the simultaneous appearance of many stressors, which leads to low feed intake, intestinal disorders, inflammation, increased incidence of disease, and mortality, which causes significant economic losses for producers [1,17]. Researchers have pointed to a specific link between mitochondrial function, oxidative stress, and weaning stress, which causes a decrease in the activity of mitochondrial respiratory complexes in intestinal and hepatic tissues during the first week after weaning [3,18]. In our study, a decrease in the concentration of total protein was found in the blood plasma of piglets of the control group on the first day after weaning. One of the reasons for this decrease in protein may be damage to protein molecules as a result of oxidative stress. Hao Y et al.[19] found that protein hydroxyl, a marker of protein oxidative damage, significantly increased in the body of piglets on the 1 day after weaning.

In another part of the same study [20], we observed that weaning piglets from sows causes oxidative stress, resulting in an increase in the concentration of metabolites associated with free radical damage. Specifically, we revealed an increase in carbonyl groups of proteins on the first day and primary metabolites of lipid peroxides on the seventh day after weaning. Our findings indicate that the activation of free radical processes in piglets occurs due to an immature antioxidant system, leading to reduced activity of the enzymatic components - superoxide dismutase, glutathione peroxidase, and catalase - in their blood, as well as a decrease in the concentration of the non-enzymatic antioxidant - reduced glutathione.

Therefore, to stimulate the body's defenses and metabolic processes in one of the most critical moments of piglets' life, we used the nettle extract. It was found that the studied extract has a positive effect on the metabolic systems of animals. The increase in morphological parameters on days 1 and 7 after weaning compared to the control indicates the hematopoietic effect of nettle, rich in trace elements, especially Iron, Zinc, and Copper, on erythropoiesis (increase in the number of red blood cells within the physiological norm), antianemia effect (increase in hemoglobin concentration) and stimulation

of respiratory function in experimental animals compared to control ones. Some authors explain the positive impact of nettle on hematopoiesis by stimulating the absorption of minerals from the feed [21-24].

The positive effect of 40% nettle extract on the hematological parameters of piglets can be explained by the action of its components. These components include ascorbic acid, vitamin E (as a part of red blood cell membranes), B vitamins, organic acids (citric, malic, succinic, etc.), and Se. These components directly or indirectly affect the oxygen transport function of hemoglobin [13,25,26]. The protein-iron complex, which is formed with a sufficient amount of Fe, helps improve absorption through the small intestinal mucosa. This is extremely important for piglets during the weaning period. It's worth noting that the increase in the number of leukocytes in the blood of piglets after weaning within the normal range under the influence of nettle may indicate its immunomodulatory properties. This is due to the presence of compounds such as quercetin, kaempferol, and isorhamnetin in the nettle extract. Some authors noted [19,27,17] that these compounds promote the rapid proliferation of bone marrow cells and the release of young blood cells into the bloodstream.

The increase in the concentration of total protein in blood of experimental piglets especially on the 1 day after weaning, confirms the activation of its synthesis under the nettle action. The high content of organic acids, such as formic, citric, and ascorbic acids, provides a positive effect of the extract on redox and anabolic processes in the body of young animals. These data are consistent with the work of A. Szewczyk in 2006, where it was found that nettle extract used as a dietary supplement for finishing pigs affects protein synthesis and fat metabolism. It also changes the profile of fatty acids and lipids in the blood, reducing total cholesterol and triglyceride concentration [28].

The positive effect of nettle extract on protein synthesis is closely related to the processes of transamination. The increase in AST and ALT activity in our study indicates the stimulation of thermogenesis and gluconeogenesis in the experimental piglets' bodies. The intensification of these processes is confirmed by an increase in the glucose concentration in the blood of piglets. This is evidence of the hydrocarbon metabolism activation under the influence of nettle extract that is necessary for the organism of young animals during weaning [2,7].

The activation of energy and redox processes under the influence of nettle can be explained by the presence of citric acid (improving the absorption of Phosphorus by the body) and silicic acid and its salts (stimulating the absorption of Calcium). A large amount of anti-oxidant compounds (ascorbic acid, carotenoids and flavonoids) in nettle also contributes to the activation of these processes [12,29].

In a previous study, we analyzed the composition of 40% of the nettle extract that was added to the diet of the experimental piglets. The concentrations of biologically active substances found were as follows: polyphenols - 11924.38 mg/100 g, carotenoids - 0.127 mg/g, chlorophyll a - 0.513 mg/g, and chlorophyll b - 0.174 mg/g.

The high activity of ALP in the blood of young animals is explained by the intensive functioning of osteoblasts in bone tissue, which is due to the processes of active growth of the organism, especially in piglets of the experimental group. During this period, the activity of the enzyme in the blood increases due to bone isoenzyme. The higher activity of ALP in the blood of experimental animals is also explained by the increased phosphorylation processes due to better intake and absorption of phosphorus and calcium (increase in the free phosphate fund by increasing ALP activity). To stimulate redox and energy processes, a high level of ATP is also required. The ATP level is provided by an increase in the activity of the CK under the nettle extract action [4,30].

The activation of anabolic processes in the organism of piglets fed with 40% nettle extract explains the increase in their live weight and average daily gain. This is also a confirmation of the positive effect of nettle extract on the general physiological state of experimental animals, compared to piglets kept on a standard diet.

The data on the elevation of piglets' weight and safety under the influence of nettle extract are consistent with the results of other authors who have observed the improvement of productivity indicators. These results indicate the increased viability, activation of metabolic and immune processes in the body, better absorption of feed and its components due to a positive effect on the gastrointestinal tract and intestinal microflora, as well as suppression of the expression of proinflammatory cytokines [1,9].

The literature analysis revealed that most studies focused on adding herbal preparations, green nettle, or a combination of other herbs, to piglet diets. Less commonly, studies used aqueous nettle extracts. The effects of nettle extracts on the body can be explained by their impact on intestinal microflora. Therefore, we focused on the anti-stress and adaptogenic effects of nettle extract on various metabolic processes. To extract the optimal amount of antioxidants to enhance the resistance of young animals under stress, we utilized a water-ethanol mixture. Our research on nettle extracts, including aqueous and ethanolic (20%, 40%, 60%, 70%, and 90%), demonstrated that the 40% extract revealed the highest radical scavenging activity. Notably, there

is limited literature data on the effect of such an extract on farm animals during weaning stress. Considering the promising outcomes, further investigation of the impact of this specific nettle extract on animals under various stressful conditions is recommended.

In conclusion, it should be noted that the addition of herbal preparations, as well as any other biologically active compounds into the diet of farm animals of different productivity and age, should be considered comprehensively and follow one rule. According to this rule the positive effect of these additives on metabolism, general resistance and safety of the organism can occur only under optimal conditions of keeping, complete and balanced feeding and ensuring a high level of their welfare.

Thus, our study has shown that a powerful complex of natural biologically active substances contained in 40% ethanolic nettle extract causes activation of metabolic processes, improved digestibility of dietary nutrients and safety of piglets during weaning. Therefore, it is suggested to add nettle extract to the standard diet of young animals to stimulate metabolic and adaptation processes, as well as improve performance indicators of their body during critical periods of ontogenesis.

Declarations

Availability of Data and Materials: The data sets analyzed during the current study are available from the corresponding author (V. Havryliak) on reasonable request.

Acknowledgments: None

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Ethical Statement: The permission to perform the experiments was obtained from the Bioethics Committee of the Institute of Animal Biology NAAS of Lviv, Ukraine, approval No 77 of 20 December, 2021.

Competing Interests: The authors declared that there is no conflict of interest.

Generative Artificial Intelligence (AI): Authors declared that the article and figures were not created by AI and AI-assisted technologies.

Authors' Contributions: O.B. designed the research and collect the samples. V.H., O.Y. performed the experimental duties of this study and analyzed the data. O.B. and V.H. wrote the first draft of the manuscript. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

References

1. Liu HY, Zhu C, Zhu M, Yuan L, Li S, Gu F, Hu P, Chen S, Cai D: Alternatives to antibiotics in pig production: Looking through the lens of immunophysiology. *Stress Biol,* 4:1, 2024. DOI: 10.1007/s44154-023-00134-w

2. Xiong X, Tan B, Song M, Ji P, Kim K, Yin Y, Liu Y: Nutritional intervention for the intestinal development and health of weaned pigs. *Front Vet Sci,* 6:46, 2019. DOI: 10.3389/fvets.2019.00046

3. Novais AK, Deschêne K, Martel-Kennes Y, Roy C, Laforest J-P, Lessard M, Matte JJ, Lapointe J: Weaning differentially affects mitochondrial function, oxidative stress, inflammation and apoptosis in normal and low birth weight piglets. *PLoS One,* 16(2):e0247188, 2021. DOI: 10.1371/journal.pone.0247188

4. Parraguez VH, Sales F, Peralta OA, De los Reyes M, Campos A, González J, Peralta W, Cabezón C, González-Bulnes A: Maternal supplementation with herbal antioxidants during pregnancy in swine. *Antioxid,* 10 (5):658, 2021. DOI: 10.3390/antiox10050658

5. Bacou E, Walk C, Rider S, Litta G, Perez-Calvo E: Dietary oxidative distress: A review of nutritional challenges as models for poultry, swine and fish. *Antioxidants,* 10 (4):525, 2021. DOI: 10.3390/antiox10040525

6. Barducci RS, Santos AAD, Pacheco LG, Putarov TC, Koch JFA, Callegari MA, Dias CP, de Carvalho RH, da Silva CA: Enhancing weaned piglet health and performance: The role of autolyzed yeast (*saccharomyces cerevisiae*) and β-glucans as a blood plasma alternative in diets. *Animals,* 14 (4):631, 2024. DOI: 10.3390/ani14040631

7. Mahfuz S, Shang Q, Piao X: Phenolic compounds as natural feed additives in poultry and swine diets: A review: *J Anim Sci Biotech,* 12 (48): 1-18, 2021. DOI: 10.1186/s40104-021-00565-3

8. Gāliņa D, Ansonska L, Valdovska A: Effect of probiotics and herbal products on intestinal histomorphological and immunological development in piglets. *Vet Med Int,* 2020:3461768, 2020. DOI: 10.1155/2020/3461768

9. Radzikowski D, Milczarek A: Selected feed additives used in pig nutrition. *J Cent Eur Agr,* 22 (1): 54-65, 2021. DOI: 10.5513/JCEA01/22.1.2927

10. Grauso L, Bruna de Falco, Lanzotti V, Motti R: Stinging nettle, *Urtica dioica* L.: botanical, phytochemical and pharmacological overview. *Phytochem Rev,* 19, 1341-1377 2020. DOI: 10.1007/s11101-020-09680-x

11. Keleş R, Şen A, Ertaş B, Kayalı D, Eker P, Şener TE, Doğan A, Çetinel Ş, Şener G: The effects of *Urtica dioica* L. ethanolic extract against urinary calculi in rats. *J Res Pharm,* 24 (2): 205-217, 2020. DOI: 10.35333/jrp.2020.137

12. Marotti I, Frassineti E, Trebbi G, Alpi M, D'Amen E, Dinelli G: Health-promoting phytochemicals of stinging nettle *(Urtica dioica* L.) grown under organic farming in Italian environments. *Ind Crop Prod,* 182 (26):114903, 2022. DOI: 10.1016/j.indcrop.2022.114903

13. Bhusal KK, Magar SK, Thapa R, Lamsal A, Bhandari S, Maharjan R, Shrestha S, Shrestha J: Nutritional and pharmacological importance of stinging nettle *(Urtica dioica* L.): A review. *Heliyon*, 8 (6):e09717, 2022. DOI: 10.1016/j.heliyon.2022.e09717

14. Morozyuk S, Protopopova V: Herbaceous Plants of Ukraine: Textbook. Ternopil: Educational Book - Bohdan, 2007.

15. Vlizlo VV (Ed): Laboratory Methods of Investigation in Biology, Stock-Breeding and Veterinary. Spolom, Lviv, 2012.

16. Walker JM (Ed): The Protein Protocols Handbook. Humana Press, Hatfield, Hertsfordshire, 2009.

17. Papatsiros VG, Papakonstantinou GI, Katsogiannou E, Gougoulis DA, Voulgarakis N, Petrotos K, Braimaki S, Galamatis DA, El-Sayed A, Athanasiou LV: Effects of a phytogenic feed additive on redox status, blood haematology, and piglet mortality in primiparous sows. *Stresses,* 4, 293-307, 2024. DOI: 10.3390/stresses4020018

18. Cao ST, Wang CC, Wu H, Zhang QH, Jiao LF, Hu CH: Weaning disrupts intestinal antioxidant status, impairs intestinal barrier and mitochondrial function, and triggers mitophagy in piglets. *J Anim Sci,* 96 (3): 1073-1083, 2018. DOI: 10.1093/jas/skx062

19. Hao Y, Xing M, Gu X: Research progress on oxidative stress and its nutritional regulation strategies in pigs. *Animals,* 11 (5): 1384, 2021. DOI: 10.3390/ani11051384

20. Buchko O, Havryliak V, Yaremkevych O, Pryimych V, Tkachuk V: The effect of nettle extract on antioxidant defense system in piglets after weaning. *Stud Biol,* 18 (1): 31-42, 2024. DOI: 10.30970/sbi.1801.756

21. Lopez-Galvez G, Lopez-Alonso M, Pechova A, Mayo B, Dierick N, Gropp J: Alternatives to antibiotics and trace elements (copper and zinc) to improve gut health and zootechnical parameters in piglets: A review. *Anim Feed Sci Tech,* 271:114727, 2020. DOI: 10.1016/j.anifeedsci.2020.114727

22. Basak G, Malakar R, Sharma A, Bharti SK, Sharma B: Medicinal herbs - Boon for modern veterinary therapeutics. *IJBSM,* 11 (1): 188-194, 2020. DOI: 10.23910/IJBSM/2020.11.2.2081b

23. De Vico G, Guida V, Carella F: *Urtica dioica* (stinging nettle): A neglected plant with emerging growth promoter/immunostimulant properties for farmed fish. *Front Physiol,* 9:285, 2018. DOI: 10.3389/fphys.2018.00285

24. Herrera SB, Rodriguez L, García MC, Flores JL, Velasco R: Effects of extract of *Urtica dioica* L. (stinging nettle) on the immune response of rats with severe malnutrition. *J Complement Med Res,* 9 (2): 63-73, 2018. DOI: 10.5455/jcmr.20180508052039

25. Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA, Kwon WB, Mathai JK, Navarro DMDL, Jaworski NW, Stein HH: Non-antibiotic feed additives in diets for pigs: A review. *Anim Nutr,* 4 (2): 113-125, 2018. DOI: 10.1016/j.aninu.2018.01.007

26. Gaykwad CK, De UK, Jadhav SE, Chethan GE, Sahoo A, Ranjan N, Mondal DB, Gaur GK, Verma MR, Chaudhuri P: Adding α-tocopherol-selenium and ascorbic acid to periparturient sow diets influences hemogram, lipid profile, leptin, oxidant/antioxidant imbalance, performance and neonatal piglet mortality. *Res Vet Sci,* 125, 360-369, 2019. DOI: 10.1016/j.rvsc.2019.07.014

27. Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL, Oh S, Gay CG: Phytochemicals as antibiotic alternatives to promote growth and enhance host health. *Vet Res,* 49:76, 2018. DOI: 10.1186/s13567-018-0562-6

28. Szewczyk A, Hanczakowska E, Świątkiewicz M: The effect of nettle (Urtica dioica) extract on fattening performance and fatty acid profile in the meat and serum lipids of pigs. *J Anim Feed Sci,* 15 (1): 81-84, 2006. DOI: 10.22358/jafs/70148/2006

29. Castillo-Velarde ER: Vitamin C in health and disease. *Rev Fac Med Hum,* 19 (4): 95-100, 2019. DOI: 10.25176/RFMH.v19i4.2351

30. Paskudska A, Kołodziejczyk D, Socha S: The use of herbs in animal nutrition. *Acta Sci Pol Zootec,* 17 (2): 3-14, 2018. DOI: 10.21005/asp.2018.17.2.01