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# Influence of Resveratrol and β-Glucan on the Aggregation of Platelets in Growing Pigs

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## Abstract

**Background:** We investigated the effect of resveratrol alone on platelet counts and ability to aggregate and its effect in combination with β-glucan.

**Methods:** The experiment included three groups of growing piglets. Resveratrol was administered to the first experimental group (R). The second experimental group (RG) received resveratrol with the addition of β-glucan. The third group was used as control (C) and received alcohol solution only. Blood samples were collected before the start of the experiment and at the end of the 1st and 2nd week of supplementation. The number of platelets, their aggregation activity (amplitude) and rate of aggregation (slope) after stimulation by ADP and Cationic Propyl Gallate (CPG) were determined.

**Results:** Resveratrol significantly reduced the numbers of platelets from 690.0 ± 145.10 × $10^9$/l detected at the beginning of the experiment, to 484.2 ± 128.50 × $10^9$/l after 2 weeks of supplementation (P<0.05). The administration of resveratrol together with β-glucan accelerated these changes; the numbers of platelets dropped significantly from the initial level of 694.8 ± 124.67 × $10^9$/l to 509.9 ± 156.28 × $10^9$/l already after one week of supplementation (P<0.05) and at the end of the experiment were 463.3 ± 195.59 × $10^9$/l (P<0.05). The aggregation activity of platelets induced by ADP decreased significantly at the end of the experiment in both experimental groups (R: P<0.01; RG: P<0.05); and was significantly reduced compared to the control group (P<0.01). Platelet aggregation rate induced by CPG was decreased in R group (P<0.05; and RG group P<0.01).

**Conclusion:** The results demonstrate the similar effect of resveratrol and β-glucan on the number of platelets and their aggregation ability in piglets.

**Keywords:** Resveratrol; β-glucan; Cationic propyl gallate; Platelet; Piglet

**Abbreviations:** ADP: Adenosine Diphosphate; CPG: Cationic Propyl Gallate; BW: Body Weight; SPAT: Slide Platelet Aggregation Test.

## Introduction

In recent years, considerable attention has been paid to the importance of resveratrol and β-glucan in human and animal nutrition. In the literature, there are a number of findings regarding the biological effects of these substances, including their impact on haematological parameters and immunity. These effects have also been studied in pigs. Glucans are a natural component of pig diet; they are part of endosperm cell walls and the subaleurone layer of grains used in feed mixtures, such as oat, wheat and barley in particular. The effects of feed supplementation with β-glucan isolated from a variety of sources such as fungi, yeasts, or kernels were the subject of investigation of many studies in the 90s and this work still continues [1-4]. We built on the results of our previous study on resveratrol; its supplementation in rats induced significant changes in the number and functional activity of platelets [5] and leukocytes [6]. In this study, we investigated the effect of resveratrol alone on platelet counts and ability to aggregate in growing piglets and its effect in combination with β-glucan.

## Materials and Methods

Three litters of 6-week-old Large White breed piglets were divided equally into a control group (C) and two experimental groups: Resveratrol group (R) and Resveratrol+β-glucan group (RG). Each group had 10 piglets that were fed *ad libitum* a complete feed mix for piglet rearing with free access to drinking water.

Dry extracts of resverin from *Polygonum cuspidatum* roots, standardised to 50% content of trans-resveratrol and its glycosides, and 6% content of emodin, which is partially soluble in water and readily soluble in ethanol, were administered daily to the piglets of group R through a gastric probe at a daily dose 3 mg/$kg^{0.75}$ b.w.

Beta-1.3/1.6 D-glucan extracted from cell wall of *Saccharomyces cerevisiae* and dry extract of resverin dissolved in ethanol were administered daily to the piglets of RG group through a gastric probe. Daily doses of resveratrol as well as β-glucan were 3 mg/$kg^{0.75}$ b.w. Also, 15% ethanol was administered daily to the piglets of C group. Experimental design was carried out according to the pattern below in Table 1.

Blood samples were collected by puncture of *V. jugularis*; 3.8% Na-citrate was used as an anticoagulant agent. Aggregation of platelets was investigated using the Bohr method by dual-channel optical aggregometer (Chrono-log, USA) with 2 inductors: ADP (Chrono-PAR ADP, Chrono-log, USA) and cationic propyl gallate (SPAT, Analytical Control Systems, Inc., USA).

Platelet aggregation was tested at a final concentration of 10 μM

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| | C<br>n=10 | R<br>n=10 | RG<br>n=10 |
|---|---|---|---|
| **Day 0** | Blood sampling 0, weight | | |
| **Day 1-6** | 20 ml of 15% alcohol per piglet per day | 25 mg of resveratrol in 20 ml of 15% alcohol per piglet per day | 25 mg of resveratrol+25 mg of β-glucan in 20 ml of 15% alcohol per piglet per day |
| **Day 7** | Blood sampling 1, weight | | |
| **Day 8-15** | 30 ml of 15% alcohol per piglet per day | 37.5 mg of resveratrol in 30 ml of 15% alcohol per piglet per day | 37.5 mg of resveratrol+37.5 mg of β-glucan in 30 ml of 15% alcohol per piglet per day |
| **Day 16** | Blood sampling 2 | | |
| **Day 17** | Weight | | |

**Table 1:** Experimental design.

ADP and at a plasma: SPAT ratio=9:1. Aggregation (%) and slope (%/min) were read from the aggregometry curve. Platelet count was performed using the haematological analyser CELLTACα (Nihon Kohden, Japan).

The health state and weight of the piglets were monitored in the same intervals of the experiment. Experiments were carried out under an institutionally-approved procedure in accordance with ethical principles and the Protection of Animals against Cruelty Act and follow-up rule. Student's t-test was used for statistical processing of all data by Microsoft Excel (mean values and standard deviation).

Experiments were carried out under an institutionally approved protocol in accordance with ethical principles and Act No. 246/1992 on the Protection of Animals against cruelty and follow-up rules.

## Results and Discussion

The number of platelets dropped after the administration of resveratrol to experimental piglets (group R) (Figure 1) from the initial value of 690.0 ± 145.10 × $10^9$/l to the final level of 484.2 ± 128.50 × $10^9$/l ($P<0.05$). These findings correlate with the previous results of Doubek et al. [5] who tested the influence of resveratrol on number and activity of platelets in experimental rats. Similarly, resveratrol also reduced number of leucocytes in piglets [6]. In our study, the number of platelets also significantly dropped after the simultaneous administration of resveratrol and β-glucan (group RG) (Figure 1). The number of platelets decreased from the initial value of 694.8 ± 124.67 × $10^9$/l to a final level of 463.3 ± 195.59 × $10^9$/l ($P<0.05$). Platelet aggregation induced by ADP was significantly reduced after two weeks in both experimental groups ($P<0.01$). In comparison to the control piglets, platelet aggregation was demonstrably lower ($P<0.01$) and the speed of aggregation also decreased significantly in both experimental groups after two weeks (C vs. R: $P<0.05$; C vs. RG: $P<0.01$) (Figures 2 and 3). These results in supplemented piglets confirmed the anti-aggregation effect of resveratrol described in previous works in laboratory animals [7-9]. Platelet aggregation induced by cationic propyl gallate was not significantly reduced (data not shown). The rate of aggregation induced by this strong inducer of aggregation decreased most markedly in group RG ($P<0.01$) (Figure 4). The results suggest that the effect of resveratrol on platelet number and activity was not inhibited by the simultaneous administration of β-glucan. On the contrary, we detected a similar effect of both agents in some of the tested parameters. These results fully correspond with the *in vitro* experiments of Saluz-Jakuszczak et al. [10,11], where β-glucan significantly decreased platelet degranulation and aggregation. Moreover, our findings also correlate with the results of Malinowska and Olas [9] who described the antithrombotic and antioxidative activity of resveratrol in similar experiments.

Platelet aggregation and rate of aggregation markedly increased in control piglets compared to those in the R and RG groups (Figures 2-4).

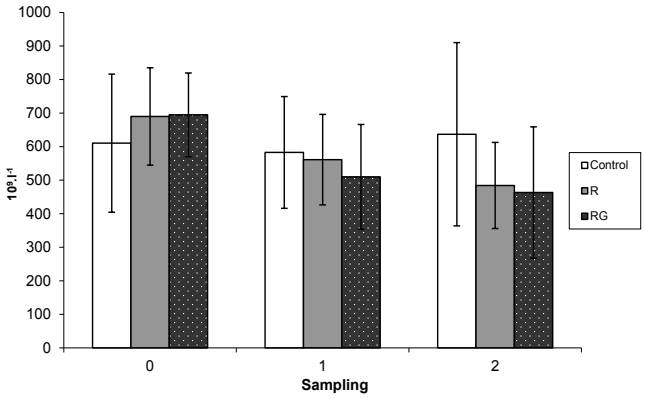


**Figure 1:** Platelet count.

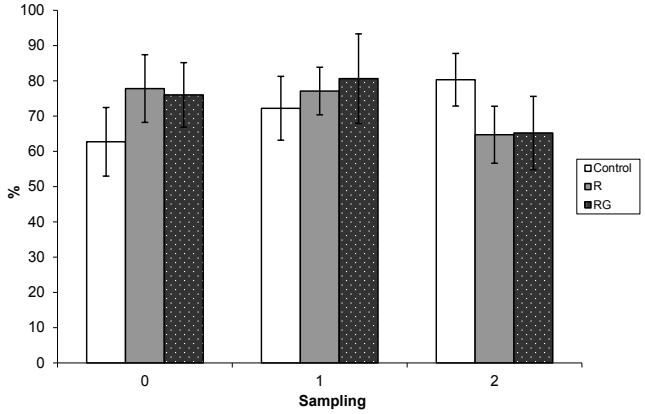


**Figure 2:** Platelet aggregation induced by ADP.

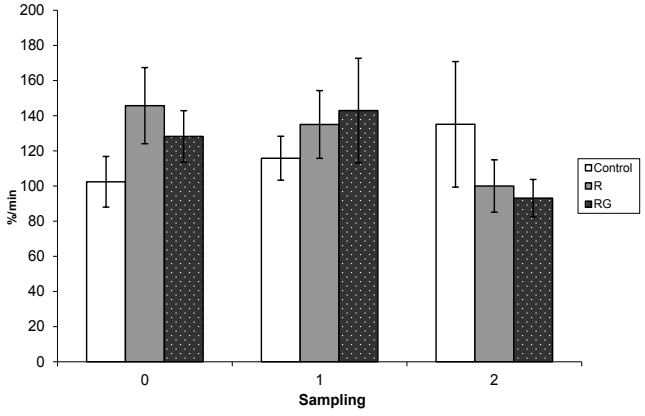


**Figure 3:** Rate of the platelet aggregation induced by ADP.

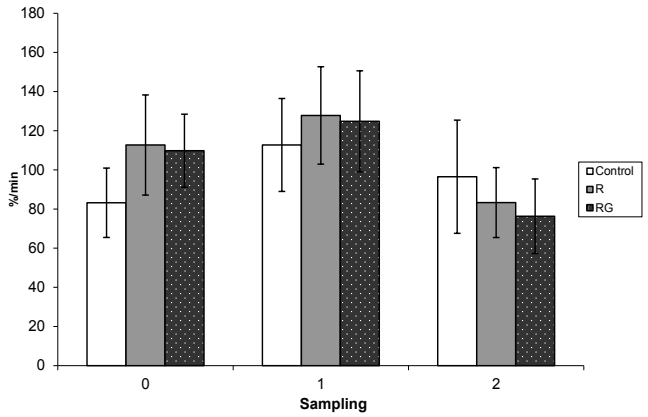


**Figure 4:** Rate of the platelet aggregation induced by propyl gallate.

If this increase is connected with stress factors such as using a gastric probe repeatedly and following blood sampling, then it can be deduced that the administration of resveratrol as well as β-glucan acts in contrast to stress in both of the supplemented groups.

Literature sources indicate that resveratrol and β-glucan are natural substances with extensive and often similar impacts on organisms. It is the result of a possible role in the production of some biologically active molecules like cytokines, mediators and growth factors [12-15]. In the literature, the effect of β-glucans on immune system and haematological parameters in piglets has been described. Ewaschuk et al. [2] detected increased erythrocyte and lymphocyte numbers and also naive T-cells in blood samples of piglets that were supplemented with graded doses of β-glucans for two weeks. In contrast with the results of Holesovska et al. [6], these authors did not prove the effect on neutrophil numbers and their activity or proliferation of incubated lymphocytes. However, they concede that stimulation *ex vivo* does not reflect conditions similar to living organisms. Contradictory data about the effect of β-glucans on haematological parameters and immunological response in piglets are not sporadic. Li et al. [4] and Wang et al. [16] emphasised that the effect of β-glucans on immune system of piglets is dependent not only on the tested substance and dosage but also on the animal's exposure to antigens. Subsequent cytokine production after such exposure, which can be supported by β-glucans, leads to the final effect. In cases where the effect of resveratrol and β-glucan in an organism is mediated via equal or comparable mechanisms, it is not surprising that we detected their synergistic effect. Similarly, Vetvicka et al. [15] assumed a synergistic effect of both substances on the immune system of mice. Thrombopoiesis, platelet release into the peripheral blood, as well as their ability to aggregate are a result of the interaction of numerous regulatory factors and also actual impacts of the environment. The exact mechanism by which resveratrol and β-glucan exert in this regulation must be determined.

## Conclusion

The results presented extend our previous findings that the so-called "aspirin-like" effect of resveratrol, which was previously demonstrated in rats, was also evident in piglets. Moreover, it can be supported by the simultaneous administration of β-glucan. Considering the piglet as a physiological model similar to humans, our results are also attractive for human medicine.

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