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ASSESSMENT OF THE EFFECT OF ALPHA-GALACTOSIDES ON YOLK SAC RESORPTION RATE IN BROILER CHICKENS

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ABSTRACT

The aim of the present study was to investigate the effect of the injection of raffinose-family oligosaccharides during embryogenesis on the rate of resorption of the yolk sac. 80 000 eggs were divided in two groups, the control and the experimental; 40 000 eggs in each. The experimental group eggs were injected on the 12th day of hatch directly into the air cell with the substance containing α–galactosides. The yolk sac's were sampled right after slaughter from earlier weighed, 1, 4, 7 and 14-day chickens, from 10 broilers each time. In the investigation the parameters of yolk sac were e.g. yolk sac/body weight ratio and the egg yolk absorption. Moreover, there was also evaluated mean body weight, feed conversion ratio, mortality rate and the European Efficiency Ratio. Results showed that the single injection have a favourable effect on yolk sac resorption rate. Yolk sac/body weight ratio decreased and the estimated yolk absorption increase in experimental group. Also better indicators of production were found in the experimental group.

Key words*:* α-galactosides, *in ovo*, poultry production, preobiotic.

INTRODUCTION

The yolk sac is the only source of nutrients for the embryo developing inside the egg. It consists of 33 % of fat, 17 % of protein, about 2% of carbohydrates, and 48 % of water (Johnson, 2000). Right after the hatch the yolk sac is internalized from the inside of the egg to the abdominal cavity where it operates as a prolongation of the intestine (Khan *et al.*, 2004). Resorbed yolk sac not only the first source of nutrients but also a source of substances taking part in the adequate development of the immune system (Noy *et al*., 1996; Wertelecki and Jamroz, 2003). The content of the yolk sac in most cases is entirely absorbed over the first 7 days after the hatch, and the yolk sac itself gets transformed into the scar tissue (Noy and Sklan, 2002; Jamroz *et al*., 2004; Uni and Ferket, 2004). However, the rate of resorption can vary and depends on many factors which regulate the process both before and after the hatch, e.g. incubation temperature, availability and type of nourishment, as well as water, right after the hatch (Khan *et al*., 2004; Lourens *et al*., 2007; Panda and Reddy, 2007). In some chickens form various reasons it happened that, the yolk sac does not undergo absorption and remains unabsorbed in the abdominal cavity. As reported by Shah *et al*. (2004), the period of the first two weeks is especially important in the life of chicks hence, it is the time when there occur 30 – 50 % of deaths of the total mortality rate. The unabsorbed content of the yolk sac can be a perfect source of nutrients for intestine pathogens, which makes it a potential reservoir of bacteria representing genus *Salmonella* (Shivaprasad, 2000), *Campylobacter*, *Escherichia coli* (Deeming, 1995; Sharada *et al*., 1999). Besides the infection can lead to the migration of microorganisms to the intestinal lumen and colonization or recolonization of the alimentary canal both before and after the hatch (Desmidt *et al*., 1997). Khan *et al*. (2004), pointed that the one of the crucial factors increasing the mortality of chickens in that period, point to the retention and infection of the yolk sac. Anjum (1997), on the other hand, reported on retention and bacterial infection of the yolk sac being the most common cause of death in chickens in Pakistan. Similarly Singh *et al*. (1993) pointed to the main cause of increased mortality being the infection of the unresorbed yolk sac. Ghodasara *et al*. (1992) observed in chickens, in connection with the infection of the yolk sac, about 31 % mortality rate. From the economic perspective, the infection of the yolk sac is one of the key diseases which significantly increases the mortality rate in the first week of life (Rad *et al*., 2003). Cortes *et al*. (2004) as well as Kizerwetter-Świda and Binek (2008) reported that the birds which would survive the infection demonstrated a low increase in the body weight and a poor carcass quality. The immune system of the chicks embryo is not mature enough to produce antibodies before the hatch, so there is no *de novo* synthesis of antibodies by the embryo (Rose and Orlans, 1981; Li *et al*., 1998). The capacity of chicks for defence from intestine pathogens in the 1st week of life is mostly based on the maternal antibodies released from the yolk sac directly to the light of the intestine and the inborn immune system (Bar-Shira *et al*., 2005; Bar-Shira and Friedman, 2006). Thus, the retention of the content of the

yolk sac can limit that process and, as a result, impair the development and the functioning of the immune system. Searching for the way to stimulate a favourable bacterial profile and metabolic processes, the authors got interested in the raffinose family of oligosaccharides (RFOs), still not long ago treated only as an antinutritious factors. However, the newly discovered properties of those compounds show that RFOs can also have a favourable effect on the organism (Guillon and Champ, 2002). Oligosaccharides representing the family of raffinose can be easily extracted and purified according the method proposed by Gulewicz *et al*. (2000), as nutrients with prebiotic effect, which are in fact a waste product in the debitering process of bitter lupin seeds. Therefore, RFOs can constitute a perfect and cheap production component of functional food (Villaluenga *et al*., 2005). Much research confirms a favourable effect of prebiotics on various aspects of health in chickens by a stimulation of the growth of beneficial intestinal microflora (Cummings and Macfarlane, 2002; Marteau and Boutron-Ruault, 2002; Xu *et al*., 2003). Villaluenga *et al*. (2004) and Pilarski *et al*. (2005) reported that administering raffinose-family oligosaccharides during the embryogenesis show a stimulating effect on the favourable bacterial profile in chickens after the hatch. In previous studies we observed significant effect of those compounds on increasing the length of the terminal ileum as well as the length of respective intestine segments in 42-day chickens (Brudnicki, 2009). Drawing on the research reviewed, the authors of the present study decided to investigate the effect of the injection of raffinose-family oligosaccharides during embryogenesis on the rate of the yolk sac resorption, which is an important aspect both from the health and from the economic perspective.

MATERIALS AND METHODS

The present study involved the use of hatching eggs from the parent flock of Ross meat line. The research included 80 000 eggs which were randomly allocated to two groups: the control group and the experimental group; 40 000 eggs in each. The experimental group eggs were injected on the 12th day of hatching directly into the air cell with the substance containing α–galactosides following the method proposed by Villaluenga *et al*. (2004). The galactosides introduced were derived as a result of the process of extraction of the residue after debitering of bitter lupin seeds (Gulewicz *et al*., 2000). After the hatch, the chickens were transferred into two litter chicken houses depending on the group they represented, with the comparable environmental conditions. The birds were fed *ad libitum* with commercial mixtures the composition of which complied with the NRC guidelines (1994). The experimental material was sampled right after slaughter from earlier weighed, 1, 4, 7 and 14-day chickens, from 10 chickens each time. After the abdominal cavity was opened, the yolk sac was dissected and weighed. On the 14th day of the experiment in both groups there was determined the percentage of the population in which the residual yolk sac was identified. Besides there was calculated the ratio of the weight of the yolk sac to the body weight (YBR) according to the following formula (Khan *et al*., 2002):

$$YBR = \frac{YSW}{BW} \bullet 100$$

where:
YSW – yolk sac weight
BW – body weight
There was also evaluated the egg yolk absorption (EYA) (Bierer and Eleazer 1965):

$$EYA = \frac{a-b}{a} \bullet 100$$

where:
a – mean yolk sac weight on the 1st day
b – yolk sac weight on the sampling day

For 42-day control and experimental chickens there were presented results of the production nutrition test concerning the following rearing indices: mean body weight (kg), Feed Conversion Ratio (FCR) (kg·kg^{-1}), mortality rate (%) and the European Efficiency Factor (EEF) calculated applying the formula by Nilipour (1998).

The results were exposed to the statistical analysis using the Student's t-test. Whenever there was no equality of variance, the Cochran-Cox test was applied. Besides, for the variables with non-normal distribution, the U-Mann-Whitney test was applied. The differences across the groups were assumed as significant at $P \leq 0.01$. The statistical analysis was made using the Statistica 7.1 statistical package.

All procedures carried out on animals were performed with the approval of the local ethical committee in Bydgoszcz.

RESULTS AND DISCUSSION

In 7-day old chickens between the groups there were reported significant differences in the body weight. In the experimental group the body weight of birds was 145.4 g and it was lower ($P \leq 0.01$), as compared with the chicken body weight in the control group 184.9 g. As for the yolk sac weight, highly differences ($P \leq 0.01$) were observed in all the groups until the 7th day of the experiment. On the 1st, 4th and on the 7th day the weight of yolk sacs in the experimental group was significantly lower. Etches (1996) report on the yolk sac after the hatch accounting for about 13.5 % of the body weight. Romanoff (1960), on the other hand, observed that the residual yolk sac accounted for about 20 % of the bird

body weight. The results of the present study, on the other hand, demonstrated that the average weight of the yolk sac in the control group of 1-day old chickens was 4.058 g, which accounted for about 9.6 % of the bird body weight, whereas in the experimental group the yolk sac weight was lower ($P \leq 0.01$) and it was 2.701 g, which accounted for about 6.4 % of the body weight (Table 1).

Table 1. Mean body weight, yolk sac weight and the estimated yolk absorption in control and experimental group

Day of life	Group	Body weight g)	Yolk sac weight g)	Yolk sac/ body weight ratio	Estimated yolk absorption %)	Retention yolk sac in the population %)
Day – 1	C control	42.4 ± 1.0	4.06 ± 0.09^{A}	9.62 ± 0.31^{A}	-	-
	E *in ovo*	41.9 ± 0.55	2.70 ± 0.08^{B}	6.45 ± 0.18^{B}	-	-
Day – 4	C control	99.4 ± 2.90	1.82 ± 0.16^{A}	1.87 ± 0.18^{A}	32.61 ± 5.84^{A}	-
	E *in ovo*	98.0 ± 2.08	0.71 ± 0.12^{B}	0.71 ± 0.12 ^{B}	73.89 ± 4.62^{B}	-
Day – 7	C control	184.9 ± 3.92^{A}	0.65 ± 0.08^{A}	0.35 ± 0.05^{A}	76.12 ± 2.89^{A}	-
	E *in ovo*	145.4 ± 5.50^{B}	0.10 ± 0.02^{B}	0.07 ± 0.01^{B}	96.41 ± 0.70^{B}	-
Day – 14	C control	405.7 ± 15.64	0.05 ± 0.04	0.01 ± 0.01	98.00 ± 1.60	30%
	E *in ovo*	349.7 ± 15.45	-	-	100	0 %

Mean ± standard error

$^{A, B}$ - different superscripts indicate significant $P \leq 0.01$) differences between groups in different days

Slightly other results were reported by Mikec *et al*. (2006) who observed that the yolk sac weight on the 1st day of the experiment was 3g. On the 7th day of the experiment the yolk sac weight in the experimental group was 0.097 g, while in the control group it was significantly higher and it was 0.645 g. The present results are similar to those reported by Wertelecki (2006) who, investigated a group of chickens fed according to the NRC recommendations, it was observed the weight of the yolk sac to be similar. It was noted that the ratio of the weight of the yolk sac to the body weight on respective days of the experiment was significantly lower in the experimental group, as compared with the control group. As for the estimated yolk absorption, the percentage of absorption was significantly higher in the experimental group on all the days of the experiment. On the 4th day of life of chicks, the percentage of yolk absorption in the experimental group was similar to the values recorded in 7-day old chickens from the control group. On the 14th day of life of birds, it was accounted for 100%, while in the control group – for 98 %; as such it was similar to the percentage of yolk absorption in the experimental group chickens on the 7th research day. Buhr *et al*. (2006), during their research observed that the content of the yolk sac remained unabsorbed and still fixed to the yolk duct in 31 % of the chickens aged 6-8 weeks. As reported by Jamroz *et al*. (2004), on the 7th day after the hatch the residual unresorbed yolk sac was found in 30 %, whereas 16 days after hatch – already in 10 % chickens.

In the present research, however, on the 14th day after the hatch there was noted unresorbed, to a different extent, content of the yolk sac in 30 % chickens in the control group. Drawing on the present research, one can formulate a hypothesis that the injection *in ovo* of α-galactosides derived from the debitering process of bitter lupin seeds affects the rate of the resorption of the content of the yolk sac, which can lead to ensuring the optimal amount of energy for chicks which have to deal with the new environmental conditions. The endodermal conditions are created by specific Immunoglobulin G (IgG) receptors, whereby the maternal IgG present in the yolk sac can be transferred to the blood vessels of the embryo with no degradation or digestion (Linden and Roth, 1978; Li *et al*., 1998). Since the yolk is the main place of the accumulation of immunoglobulins, due to an increased rate of yolk resorption, one can expect their earlier transfer both prior to and after the hatch. As for the retention or infection of the yolk sac, the process is disturbed. The yolk material is transported in two ways; directly into the circulation via the blood vessels of the yolk sac and into the intestine via the yolk duct (Noy *et al*., 1996). Short Chain Fatty Acids (SCFA) as the products of bacterial fermentation, can affect the process of resorption both by the acceleration of the blood flow in the mucosa and by the effect on the intestinal peristaltic. Besides, the SCFAs produced in intestines and absorbed to the blood circulation affect not only the muscles of the terminal ileum but also the small intestine, which is essential for maintaining the functions of the entire gastrointestinal tract, not only the large intestine. It is expected that a greater blood flow increases the oxygenation to the cells and the transport of absorbed nutrients, also those which originated from the yolk sac.

The application of a single injection on the 12th day of the embryonic development demonstrated a favourable effect on the rearing indices recorded during the production nutrition test. As compared with the control group, in the experimental group (*in ovo*) there was noted a higher mean body weight, lower mortality

rate and a better use of feed per body weight unit (Table 2).

Table 2. Productive indices of different groups tested at the end of growing period 42days)

Group	Body weight (kg)	Mortality ratio (%)	FCR	EEF
Control	2.17	2.73	1.85	267
In ovo	2.30	2.54	1.78	290

The EEF was 23 points higher in the experimental group injected *in ovo*. It should be pointed out that the data recorded for the greater body weight and a lower mortality rate in the experimental group birds. In the chicks in which there is no absorption of the residual yolk sac or it is disturbed or the chicks have no direct access to animal feed, the period of transferring from the environment *in ovo* to the functioning beyond it gets much longer (Sklan, 2003). Therefore, an increased rate of resorption of the yolk sac can shorten that critical period. The residual yolk sac plays the key role in supplementing the absorbed nutrients to ensure a fast growth of chicks after the hatch. As reported by Henderson *et al*. (2008), the dependence of the chicks on the content of the residual yolk sac over the first few days after the hatch limits the growth potential of broiler chickens. The total and fast resorption of the yolk sac as a component of optimal nutrition in the critical neonatal period can constitute another tool to enhance the effectiveness of poultry production. As for mass production, even a slight unitary effect is extremely important and so the present results are important, mostly from the economic point of view. Although, the mechanism whereby the injection *in ovo* of galactosides accelerates the resorption of the content of the yolk sac and the related implications require further research.

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