Assessment of the influence of oligosaccharides isolated from pea seeds on functional quality of quail

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ABSTRACT: An experiment was conducted on mature Pharaoh quail kept in cages with constant access to feed and water. The birds were divided into three feeding groups (two replications) of 48 female and 16 male birds each. Quail of the first group were fed a standard feed, those of group 2 and 3 received feed enriched with oligosaccharides at a dose of 0.4 g and 3 g, respectively. In the course of the experiment the following measurements were taken: bird weight, feed intake, egg laying capacity, egg weight, death rate, egg hatchability. Blood was examined for cholesterol and triglycerides, yolks for the protein and gammaglobulin level. The digestive tract was analysed for bifidobacteria counts. The oligosaccharide-enriched feed reduced the time of maturation, increased egg laying capacity and egg weight, and also decreased the consumption of feed per egg. No clear influence of the oligosaccharide supplementation was found as far as the blood cholesterol and triglyceride content was concerned and gammaglobulin in the eggs. The quail of the groups receiving oligosaccharides had lower bifidobacteria counts in their digestive tracts.

Keywords: quail; pea; oligosaccharides; bifidobacteria; egg; immunoglobulin; cholesterol

Oligosaccharides are commonly found in the plant kingdom. Rich in these compounds are particularly seeds of pulse plants, taking part in vital processes. Scarce studies on the influence of oligosaccharides on animals indicate that their positive effect is due to their beneficial action towards changes of the intestinal microflora. The numbers of *Bifidobacterium* and *Lactobacillus* increase, whereas those of pathogenic bacteria in the colon decrease, since they are bound with the mannose of oligosaccharides, thus leaving the digestive tract with undigested remnants. Some researchers used oligosaccharides for the purpose of *Salmonella* eliminating from the digestive tract, and in this way also eliminating the bacteria from poultry products (Bailey *et al*., 1991; Waldroup *et al*., 1993; Choi *et al*., 1994). Savage and Zakrzewska (1996) found that an addition of some oligosaccharides stimulated the immune system of birds, increasing their resistance to diseases. It has also been shown that as an effect of oligosaccharides the content of toxic metabolites and harmful enzymes decreases. Terada *et al*. (1994), who added oligosaccharides to broiler chicken feed, found an increased concentration of volatile fatty acids, and a decreased content of putrid products, such as phenol, p-cresol and ammonia in faeces. There are also reports indicating that fructooligosaccharides can be regarded as feed digestibility enhancing additives (Ammerman, 1988). Tarasewicz (1998) and Savage and Zakrzewska (1996) found that a feed enriched with oligosaccharides improved some quality indexes of the product.

It should be stressed that the knowledge of the influence of oligosaccharides on the digestive tract is still limited, and research results are often divergent (Jamroz *et al*., 1997; Tarasewicz, 1998).

The use of proper oligosaccharides can be regarded as an element of the strategy aimed at elimination of feed antibiotics, which are socially rather unacceptable because of the danger of antibiotics residue presence in animal products or in the environment.

Considering the fact that due to the chemical composition of the subunits, their linking, length and type of the chain, the influence of oligosaccharides on the digestive tract, microbial composition within the colon, and fermentation processes can vary, this study was another attempt to determine the dose of oligosaccharides isolated from pea seed, added to feed of adult quail.

MATERIAL AND METHODS

The biological assay of pea oligosaccharides was done on adult quail of the Pharaoh breed, kept in cages, under optimum microclimate conditions, according to a technology for this kind of poultry. The quail (144 females and 48 males), obtained from own hatch and raising, were divided into three groups, in two replications, according to the feed given. A full-dose mixture for adult quail was given that had been prepared in compliance with the directives for poultry feeding (Feeding Norms for Poultry, 1996), by the dose system. The first group was the control, groups two and three were the experimental groups which received feed enriched with oligosaccharides at a dose of 0.4 and 3.0 grams per 1 kg of mixture. The sugar fraction of the pea extract was prepared in accordance with the method developed by the Institute of Bioorganic Chemistry, Polish Academy Science in Poznan. Alpha-galactosides were isolated from *Pisum sativum* L. cv. Opal seeds according to the method described by Gulewicz *et al*. (2000). The analysis of separation and quantification of pea raffinose family oligosaccharides (RFOs) was performed by high-performance liquid chromatography using a refraction index detector (HPLC-RI). The chromatographic system was controlled by a PC with Maxima HPLC system controller software. A precolumn (3.2 mm i.d. × 4.0 cm) packed with C18 Porasil B and u-Bondapak/carbohydrate column (3.9 mm i.d. × 30 cm) were applied. The mobile phase was acetonitrile/distilled water (75 : 25 v/v). The flow rate was 2.0 ml/min. Solvents were filtrated through a Millipore FH (0.45 μm) membrane and degassed under helium. Injection volume was 100 μl Standard Solution Preparation.

Different amounts of raffinose and stachyose were dissolved in distilled water. Acetonitrile was added to each solution to obtain a composition similar to that of the mobile phase (75 : 25 v/v). Quantification of each sugar was accomplished by comparing the peak areas of the samples with those of the standard solutions. A standard curve was plotted for each sugar and adjusted using the least-squares method. The regression coefficients of the curves for raffinose and stachyose were always higher than 0.990.

Average content of the particular alpha-galactosides and sucrose in pea preparation (%): sucrose 18.30, raffinose 5.00, stachyose 52.50, verbascose 20.50, total 96.30.

The method of obtaining the oligosaccharides from pulse crop seeds allows to get a pure fraction whose solid state enables easy mixing with pulverised feed.

During the experiment the following measurements were done: body weight at the 5th and 18th week of life, daily feed intake, egg laying recording, egg weight, bird mortality and egg hatchability. The feed conversion per egg was also calculated.

During the decapitation of the birds, blood samples were taken in order to analyse possible morphological changes; some organs (heart, liver, stomach) were also collected in order to determine their relative weight, thus to compare the variability of these organs resulting from different feeding. In order to count bifidobacteria, bowel samples were also taken (150–180 mg) from the sites where the jejunum meets the ileum – at the level of caecum. The samples were kept in sterile plastic bags and in an airtight container with dry ice in CO_2 atmosphere at –70°C.

Lithium chloride-enriched MRS medium, a selective factor, was applied for testing the effects of the saccharides on the growth of selected *Bifidobacterium* strains. All components were diluted in distilled water and the medium of pH 6.5 was sterilised at 121°C for 15 minutes. The bacteria were counted indirectly. For this purpose, the selective MRS medium was inoculated with 1 cm^3 of 10-fold dilutions. Incubations were carried out in anoxic conditions (CO_2 atmosphere) for 72 hours at 37°C.

The chemical blood analyses were done using ready-made kits for spectrophotometry. The concentrations of total cholesterol, high-density lipoprotein (HDL), triglycerides, uric acid and glucose were determined by enzymatic methods using kits produced by BioMerieux (France). Total proteins were determined by the burette method using Biomerieux reagents.

In egg yolks the protein analysis was done by electrophoresis (reagents by Cormay), and read by a densitometer of the same manufacturer.

The results were analysed statistically by the one-way analysis of variance and Duncan's test.

RESULTS AND DISCUSSION

The quail flock of the Pharaoh breed studied for 12 weeks was fed a feed mixture the composition of which met the standards for quail in Feeding Norms for Poultry, 1996 – Table 1. Energy value of the feed (11.72 MJ) and protein content (20.7%) met the theoretical assumptions. The quail have a more intense metabolism than the other poultry species, hence the need for using a feed of a high nutritional value. Despite a higher content of fibre, the nutritional value of the used feed was close to their food requirement.

The weight of 5-week-old quail of both sexes was similar in all groups and ranged from 193.7 g (group 2) to 203.0 g (group 3) for females and from 173.5 g (group 3) to 174.2 (group 2) for males, the differences not being significant (Table 2). Pudyszak

Table 1. Composition of feed for adult quail (%)

Ingredient	Proportion	Ingredient	Proportion
Ground wheat	30.00	Lysine	0.20
Ground triticale	20.00	Methionine	0.03
Ground barley	11.67	**Chemical composition**	
Extracted soybean meal (46%)	19.20	Dry matter	85.47
Extracted rapeseed meal (35.5%)	5.00	Total protein	20.70
Meat and bone meal (55%)	5.00	Crude fibre	4.03
Poultry fat	2.00	Ether extract	5.26
Rapeseed oil	1.60	Ash	5.75
NaCl	0.20	Ca	2.84
Limestone	3.20	P	0.93
Dicalcium phosphate	0.70	Metabolizable energy (MJ)	11.72
Premix	1.20		

Table 2. Performance traits of quail until 18 weeks of age ($\bar{x} \pm$ SD)

Specification	Group		
	I (control)	II*	III**
Sexual maturity (days)	49	50	46
50% egg laying (days)	62	62	49
Egg laying (%)	86.1	83.9	90.1
Egg weight (g)	$12.1^{a} \pm 0.45$	$12.6^{b} \pm 59$	$12.3^{ab} \pm 0.56$
Feed intake (g/individual/day)	$30.1^{a} \pm 1.40$	$29.9^{a} \pm 1.60$	$29.7^{a} \pm 1.30$
Feed conversion (g/egg)	44.9	45.4	42.4
Body weight at 5th week of life (g)			
female	$201.4^{a} \pm 20.1$	$193.7^{a} \pm 11.5$	$203.0^{a} \pm 14.8$
male	$173.7^{a} \pm 12.5$	$174.2^{a} \pm 16.1$	$173.5^{a} \pm 11.7$
Body weight at 18th week of life (g)			
female	$225.5^{a} \pm 23.3$	$234.0^{a} \pm 21.0$	$231.3^{a} \pm 32.7$
male	$196.8^{a} \pm 18.6$	$207.2^{a} \pm 22.1$	$198.8^{a} \pm 8.32$
Mortality (%)	0	0	0

*0.4 g oligosaccharides per 1 kg of feed; **3 g oligosaccharides per 1 kg of feed

^{a,b}means with different letters differ significantly ($P < 0.05$)

and Mikulski (1998) reported a lower weight of 6-week quails of the same breed – the difference being 30 g. In the final stage of the experiment, the highest value of this trait was shown by females and males of group 2, respectively 234.0 and 207.2 g, whereas the lowest by group 1 (225.5 and 196.8 g). A positive influence of dietary oligosaccharides on weight gain was found in our earlier studies (Tarasewicz, 1998; Tarasewicz *et al*., 1998) and also in the research by Ammerman (1989) and Waldroup *et al*. (1993).

The quail of the 3rd group reached their sexual maturity in 46 days, i.e. by 3 and 4 days earlier than those of group 1 and 2 (Table 2). Twelve days from the egg laying onset, the flock's productivity was about 50%. According to Shanawany (1994) the period of full maturation, that is when the flock achieves 50% of egg laying capacity, depends on the day length. Sakurai (1983), who kept the flock in the regime 16L : 8D, reported that the 50% egg production was reached at 55 days of age, whereas in a short daylight regime (8L : 16D) at 70 days of age. Nagarajan *et al*. (1991) concluded that the area per one bird also influenced the period of sexual maturation in a quail flock. Quail studied by Tarasewicz (1998), who also investigated into the effect of oligosaccharides on Pharaoh quail performance, began and completed their maturation a little earlier.

A positive effect of oligosaccharides on egg production was also found although only in the group which was fed the diet with elevated oligosaccharides content, that is 3 g/kg feed. The highest egg laying capacity calculated for the 12-week laying period was in group 3 (90.1%), the control group had only 86.1% (Table 2). The egg weight was recorded during the whole period of egg laying. In groups that received oligosaccharides it was between 12.3 g (group 3) and 12.6 g (group 2), as

Table 3. Fertilization and hatchability indexes of quail eggs ($\bar{x}$ ± SD)

Specification	Group		
	I (control)	II*	III**
Egg weight (g)	$11.9^a \pm 0.94$	$11.5^a \pm 0.83$	$11.8^a \pm 0.63$
Fertility (%)	86.7	86.7	86.7
Hatchability (%)			
eggs set	56.6	76.6	66.6
fertilized eggs	65.4	88.5	76.9
Weight of 1-day chick (g)	$7.8^a \pm 8.7$	$7.2^a \pm 0.4$	$7.6^a \pm 0.46$

*0.4 g oligosaccharides per 1 kg of feed; **3 g oligosaccharides per 1 kg of feed
[a]means in rows marked with the same letters do not differ significantly ($P < 0.05$)

Table 4. Some chemical indexes of quail blood ($\bar{x}$ ± SD)

Specification	Group		
	I (control)	II*	III**
Protein (g/l)	$34.57^a \pm 4.67$	$36.98^a \pm 3.93$	$37.93^a \pm 3.04$
Glucose (mmol/l)	$14.76^a \pm 4.49$	$14.87^a \pm 2.09$	$15.59^a \pm 1.84$
Cholesterol (mmol/l)	$4.92^a \pm 1.42$	$4.88^a \pm 0.94$	$4.37^a \pm 0.69$
HDL (mmol/l)	$1.94^a \pm 0.45$	$1.98^a \pm 0.28$	$1.87^a \pm 0.44$
Triglycerides (g/l)	$9.73^a \pm 3.49$	$10.91^a \pm 3.02$	$8.46^a \pm 2.39$
Uric acid (μmol/l)	$176.91^a \pm 46.3$	$348.7^b \pm 159.8$	$352.9^b \pm 165.1$

*0.4 g oligosaccharides per 1 kg of feed; **3 g oligosaccharides per 1 kg of feed
[a, b] means with different letters differ significantly ($P < 0.05$)

compared to 12.1 in group 1 (Table 2). Significantly larger eggs were laid in the group whose egg laying index was the lowest. Ammerman (1988) described the improvement of poultry performance as an effect of oligosaccharides added to their feed. According to this author, this probiotic can be regarded as a substitute for subtherapeutic doses of antibiotics, egg laying stimulators that are unacceptable in farm poultry feeding. The daily intake of feed by quail was similar in all groups, though if expressed by absolute figures it was a little lower in the experimental groups than in the control group (Table 2). The lowest feed amount per egg was in group 3 (42.4 g), that means in the group of the highest egg laying capacity, which confirmed that the higher that capacity, the lower the feed consumption per production unit (Table 2). Tarasewicz (1998), who studied the influence of lupine oligosaccharides on quail reproductive traits, reported similar results. The daily feed intake by one bird and its utilisation for egg production depend on the energy value of the diet. Yamane *et al*. (1980), who compared feeds

Table 5. Composition of quail egg-yolk protein ($\bar{x}$ ± SD)

Specification	Group		
	I (control)	II*	III**
Total protein (g/l)	35.3	41.8	32.6
Prealbumin	$0.70^a \pm 0.39$	$1.64^a \pm 2.51$	$1.04^a \pm 0.68$
Albumin	$6.43^a \pm 2.63$	$7.36^a \pm 3.11$	$5.01^a \pm 1.46$
α_1-globulin	$0.96^a \pm 0.33$	$0.78^a \pm 0.43$	$0.62^a \pm 0.36$
α_2-globulin	$15.88^a \pm 5.69$	$18.11^a \pm 9.26$	$14.46^a \pm 5.16$
α_3-globulin	$2.45^a \pm 1.08$	$1.68^b \pm 1.89$	$1.85^b \pm 1.22$
β_1-globulin	$3.13^a \pm 1.90$	$5.34^a \pm 2.90$	$4.59^a \pm 2.68$
β_2-globulin	$2.45^a \pm 3.09$	$3.93^a \pm 2.51$	$1.74^a \pm 1.18$
γ-globulin	$1.72^a \pm 1.43$	$3.00^a \pm 7.07$	$3.32^a \pm 3.04$
Albumin/globulin	3.74	2.45	1.51

*0.4 g oligosaccharides per 1 kg of feed; **3 g oligosaccharides per 1 kg of feed
$^{a, b}$means with different letters differ significantly ($P < 0.05$)

Table 6. Weight of some internal organs and their proportion in body weight ($\bar{x}$ ± SD)

Specification	Group		
	I (control)	II*	III**
Body weight (g)	$210.2^a \pm 9.08$	$214.1^{ab} \pm 14.09$	$232.1^c \pm 19.18$
Heart weight (g)	$1.80^a \pm 0.18$	$1.91^a \pm 0.23$	$1.93^a \pm 0.28$
(%)	0.86	0.89	0.83
Liver weight (g)	$6.22^a \pm 0.56$	$6.48^a \pm 1.20$	$5.70^a \pm 079$
(%)	2.96	3.03	2.45
Stomach weight (g)	$3.48^a \pm 0.60$	$3.63^{ab} \pm 0.47$	$4.16^b \pm 0.71$
(%)	1.66	1.64	1.79

*0.4 g oligosaccharides per 1 kg of feed; **3 g oligosaccharides per 1 kg of feed
$^{a, b, c}$means with different letters differ significantly ($P < 0.05$)

of various energy values (from 9.5 to 13.3 MJ/kg), reported a daily feed intake from 22.0 to 19.8 g per bird, and from 21.5 to 38.2 g per egg. The higher the energy value of the feed, the lower its intake and consumption per unit of production.

In the course of the experiment no losses and no culling due to illness occurred. The few papers dealing with the influence of oligosaccharides on birds proved that these substances had a beneficial effect, improving health and productivity of the birds (Newman, 1995; Savage and Zakrzewska, 1996).

An analysis of the hatching process showed that the fertilization index was similar in all groups (86.7% – Table 3). The best hatching indexes from set and fertilized eggs were found in group 2 – 76.6% and 88.5%. In group 3 the percentage of hatching was by approx. 10% lower. The worst hatching indexes were obtained in the control group, only 56.6 and 65.4%. Similar results were reported by Tarasewicz (1998).

As can be seen in Table 4, there was no significant influence of oligosaccharides on the content of protein or sugar in quail blood. The lowest cholesterol content was in group 3 (4.37 mmol/l). These data correspond to some extent to the results obtained by other authors (Tarasewicz, 1998; Siegel *et al*., 1995), who reported rather a sharp decrease of cholesterol in blood as an effect of oligosaccharide-enriched diet. These sugars remain longer in the large intestine, thus they enhance the excretion of bile acids caused by volatile fatty acids production. As a result of this process the level of cholesterol in blood decreases (Hidaka, 1991; Terada *et al*., 1994). Volatile fatty acids and products of their metabolism also decrease the production of endogenous cholesterol. The cholesterol content in quail blood depends also on the bird's age (Hammad *et al*., 1995). No statistically significant differences were found in the content of HDL fraction and triglycerides in the blood collected from quail fed diets with various supplements of pea-isolated oligosaccharides and without them. On the other hand, a significantly higher level of uric acid was found in the blood of quail from both experimental groups (348.7 and 352.9 μmol/l). Uric acid, as it is well known, is a final product of protein metabolism in birds and represents the main part of redundant nitrogen eliminated from the organism. It is possible that oligosaccharides resulted in better protein digestibility. The highest concentration of total protein in yolk was found in group 2 (41.8 g/l), the lowest in group 3 (32.6 g/l), with 35.3 g/l in the control group (Table 5). These results indicate that the influence of a low dose of oligosaccharides on protein content in egg yolk is positive. No significant differences in albumin and globulin contents were found, although a clear difference, in absolute figures, was noticed in gammaglobulin content. The IgG of birds, often defined as IgY, is the only immunoglobulin of birds that is transferred from mother to offspring and is responsible for the bird's natural passive immunity. Egg yolk could become an inexpensive source of gammaglobulin, and obtained non-surgically. Besides the egg yolk immunoglobulin, egg might be used as a source of other pharmaceutics. Nowadays, another substance of high biological activity can be obtained from eggs, i.e. lysozyme. This enzyme has antibacterial, antiviral and anti-inflammatory properties.

An analysis of the proportion of various organs in relation to body weight of quail, which depicts their growth, showed that the applied oligosaccharides had no significant influence on the weight of heart and liver, whereas the weight of stomach was higher in quail of group 3 (4.16 g), which received the highest dose of oligosaccharides (Table 6). A reversed relationship between stomach weight and oligosaccharides was observed by Tarasewicz (1998). It should be stressed that the relations between animal weight and weight of their internal organs on the one hand and dietary probiotics on the other were dealt with in very few studies, hence the discussion of this matter is rather difficult.

The highest *Bifidobacterium* cell count in 1 g of intestine was found in the control group, and it differed significantly from that of both experimental groups (Figure 1). Of the two groups that were

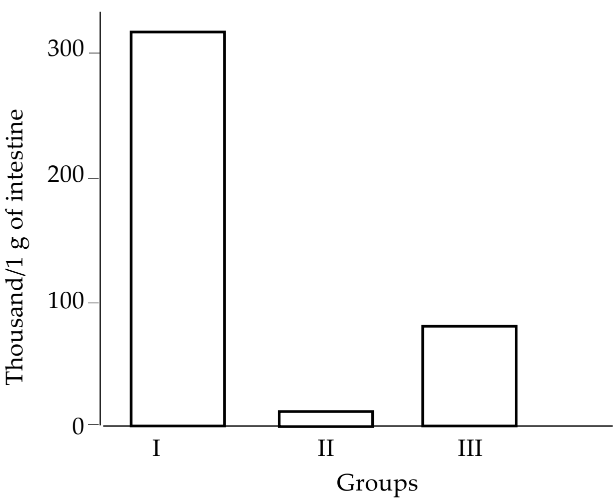

Figure 1. Counts of bifidobacteria in 1 g of intestine

fed oligosaccharides, higher *Bifidobacterium* counts were found in group 3, in the group that received the highest dose of oligosaccharides.

The positive picture of bifidobacteria counts resulting from dietary oligosaccharides was confirmed by the few research reports (Tomamatsu, 1994; Tarasewicz, 1998). These authors stressed the fact of a higher level of fat-decomposing enzymes present in the posterior section of the digestive tract, as well as of those participating in carbohydrate breakdown. Because of that, the remains of feed which have not been digested by enzymes of the pancreas, intestines and bile, are digested by the enzymes of the bacteria present in the posterior part of the digestive tract, hence the digestibility of the feed increases (Newman, 1994).

CONCLUSIONS

1. Oligosaccharides isolated from pea seeds and added to quail feed shortened the time of reaching their maturity, enhanced egg laying, increased egg weight, and decreased the intake of feed per egg.
2. Improved hatchability indexes were found in birds receiving oligosaccharides.
3. The oligosaccharides had no clear impact on the content of cholesterol and triglycerides in quail blood while the level of uric acid was significantly higher. The quail of the groups receiving oligosaccharides had lower bifidobacteria counts in their digestive tracts.

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ABSTRAKT

Hodnocení vlivu oligosacharidů izolovaných ze semen hrachu na funkční kvalitu křepelek

Pro pokusy byly použity pohlavně dospělé křepelky plemene Farao, které byly chovány v klecích se stálým přístupem ke krmivu a vodě. Zvířata byla rozdělena do tří skupin (se dvěma opakováními), v každé skupině bylo 48 slepiček a 16 kohoutů. Křepelky v první skupině byly krmeny standardní krmnou směsí, zatímco křepelky ve druhé a třetí skupině dostávaly krmnou směs obohacenou o oligosacharidy v dávce 0,4 g resp. 3 g. V průběhu pokusu byla zjišťována hmotnost křepelek, příjem krmiva, snáška, hmotnost vajec, mortalita a líhnivost vajec. V krvi byly zjišťovány hladiny cholesterolu a triglyceridů, ve žloutku obsah proteinů a hladina gamaglobulinu. V trávicím traktu byl měřen počet bifidobakterií. Podáváním krmné směsi obohacené o oligosacharidy došlo ke zkrácení doby dospívání, zvýšení snášky a hmotnosti vajec a snížení spotřeby krmiva (vztaženo na jedno vejce). Nebyl zaznamenán jednoznačný vliv přídavku oligosacharidů na obsah cholesterolu a triglyceridů v krvi a na obsah gamaglobulinu ve vejcích. Křepelky krmené dietou obohacenou o oligosacharidy vykazovaly v trávicím traktu nižší počty bifidobakterií.

Klíčová slova: křepelky; hrách; oligosacharidy; bifidobakterie; vejce; imunoglobulin; cholesterol

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