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# EFFECT OF PROBIOTICS AND AVOTAN ON THE LEVEL OF THYROID HORMONES IN THE BLOOD PLASMA OF BROILER CHICKENS

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## Abstract

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A feeding experiment was conducted to evaluate the effects of Lacto Sacc, Yea Sacc and Avotan in the diets on the level of thyroxin, triiodothyronine and thyrotrophin hormone in the blood plasma of broiler chickens. Six hundred day old commercial broiler chickens were weighted and assigned randomly in four dietary treatments: 1. a basal diet (control); 2. a basal diet + 0.1 % Lacto Sacc ; 3. a basal diet + 0.1 % Yea Sacc ; 4. a basal diet + 0.01 % Avotan. Each dietary treatment had three replicates with 50 broiler chickens per replicate.

The quantity of thyroxine did not change significantly in the supplementation of 0.1 % Lacto Sacc, 0.1 % Yea Sacc and 0.01 % Avotan in the diets of broiler chickens.

The level of triiodothyronine in the blood plasma increased insignificantly with the supplementation of 0.01 % Avotan, while this level significantly increases in the supplementation of 0.1 % Lacto Sacc and 0.1 % Yea Sacc in the diets of broiler chickens.

Plasma thyrotrophin levels were not significantly different between untreated and treated broiler chickens.

*Key words*: probiotics, AVOTAN, broiler chickens, thyroid hormones

## Introduction

Probiotics are feed supplements, which beneficially affect the health and the performance of broiler chickens by improving the intestinal microflora (Fuller, 1999; Panda et al., 2000; Chotinsky et al., 2003).

The intestinal microflora has a significant influence on the endocrinal function of animals. This has been found in comparative studies of the conventional and germ free animals (Ohsawa et al., 1981). Ukai and Mitsuma (1981) have demonstrated in germ free rats the low level of thyroxine and high level of triiodothyronine in the blood plasma as compared to conventional rats. It has also been found that iodine intake decreases in the germ free and treated with canamycin rats (Vought et al., 1972).

The experimental evidence of the effect of antibiotics on the thyroid gland is not equivocal. Ali (1983) determined that the weight of the adrenal gland and the thyroid gland increased with the supplementation of 0.04 % furazolidon for 10 days in the diets of chickens. In rats, the supplementation of chlortetracycline, carbadox, bambermycin or copper can only release limited amounts of cortisone, triiodthyronine and thyroxine respectively before activation in a counter regulatory way within 14 to 21 days (Peters, 1990). According to Andersson and Gary - Andersson (1973) however, the inclusion of amprolium in the diet significantly decreased the thyroid activity. These authors also reported that payzone caused more pronounced retardation of thyroid activity in growing chickens.

In conventional animals, the pathogenic microorganisms and antibiotics are the direct factors, which change the intestinal microflora. Indirectly the intestinal microflora may be changed also with the stressing of the animals (Suzuki et al., 1983).

Lately, great attention was given to the use of biological substances (probiotics) which influenced favorably the growth of animals and depressed the development of the

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pathogenic microorganisms (Rada et al., 1995; Helander et al., 1997; Panda et al., 2000; Chotinsky et al., 2003). The use of probiotics in order to exclude competitively the colonization of the pathogenic microorganisms has been proposed, especially after the ban of the European Committee of some nutritive antibiotics.

There is few information on the effect of probiotics and nutritive antibiotics on the level of thyroid hormones in the blood plasma of animals.

The aim of the present study was to evaluate the effect of Lacto Sacc, Yea Sacc and Avotan on the level of thyroid hormones in the blood plasma of broiler chickens.

## Material and Methods

***Animals and diets***

Six hundred day old commercial broiler chickens were wing banded, weighted and distributed randomly into four groups, each comprising three replicates of 50 birds each. Each replicate was kept in a separate floor pen on wood shavings litter. All broiler chickens were kept under uniform management condition during the test period and fed seven weeks with mash based diets (Table 1) supplemented (without control group ), 0.1 % Lacto Sacc, 0.1 % Yea Sacc and 0.01 % Avotan. Feed and water were supplied *ad libitum* throughout the experimental period.

***Probiotics***

Lacto Sacc (Alltech) contained three strains of variable organisms namely Lactobacillus acidophilus at least 100 million colonies forming units per g; *Saccharomyces cervisae* 2 x $10^{11}$/g and *Streptococcus faecium* at least 74 million colonies forming units g.

Yea Sacc (Alltech) contained Saccharomyces cervisae $10^8$/g.

***Sample procedure and analysis***

At the end of the experiment, six male broiler chickens from each dietary treatment were selected and blood samples were collected into tubes coated with heparin and then centrifuged at 4000 rotation for 10 min to isolate the blood plasma. The blood plasma was separated and stored at – 20°C until it was analyzed.

Plasma concentrations of thyroxine ($T_4$), triiodothyronine ($T_3$) and thyroid stimulating hormone (thyrotrofic hormone)

**Table 1**
**Ingredient composition of the experimental diets, %**

| Ingredients | Groups | | | |
|---|---|---|---|---|
| | Control group | Avotan | Lacto Sacc | Yea Sacc |
| Corn | 55.632 | 55.622 | 55.532 | 55.532 |
| Sunflower meal | 5.000 | 5.000 | 5.000 | 5.000 |
| Soybean meal | 33.700 | 33.700 | 33.700 | 33.700 |
| Tricalcium phosphate | 1.700 | 1.700 | 1.700 | 1.700 |
| Limestone | 1.000 | 1.000 | 1.000 | 1.000 |
| Salt | 0.250 | 0.250 | 0.250 | 0.250 |
| Premix | 0.500 | 0.500 | 0.500 | 0.500 |
| Monensin | 0.100 | 0.100 | 0.100 | 0.100 |
| DL-metionine | 0.100 | 0.100 | 0.100 | 0.100 |
| Antioxidant | 0.018 | 0.018 | 0.018 | 0.018 |
| Fat | 2.000 | 2.000 | 2.000 | 2.000 |
| Avotan | - | 0.010 | - | - |
| Probiotic | - | - | 0.100 | 0.100 |
| Total | 100.000 | 100.000 | 100.000 | 100.000 |
| Crude protein, % | 21.53 | 21.53 | 21.52 | 21.52 |
| ME, kcal/kg | 2915 | 2915 | 2912 | 2912 |
| Lysine, % | 1.14 | 1.14 | 1.14 | 1.14 |
| Methionine, % | 0.48 | 0.48 | 0.48 | 0.48 |
| Meth. + cystine | 0.82 | 0.82 | 0.82 | 0.82 |
| Ca, % | 0.94 | 0.94 | 0.94 | 0.94 |
| P (available), % | 0.46 | 0.46 | 0.46 | 0.46 |

(TSH) were measured in all blood samples using radioimmunoassay (RIA) kits

***Statistical analysis***

Statistical analysis of the level of thyroid hormones was performed by analysis of variance. All significant differences were at P<0.05.

## Results and Discussion

The level of thyroxine, triiodothyronine and thyroid stimulating hormone in the blood plasma of broiler chickens fed different ration in the experiments was summarized in Figures 1, 2 and 3.

The results indicated that the quantity of thyroxine in the blood plasma did not change significantly with the supplementation of 0.01% Avotan, 0.1% Lacto Sacc and 0.1% Yea Sacc in the diets (Figure 1).

The level of triiodothyronine in the blood plasma increased insignificantly with the supplementation of Avotan in the diets (Figure 2). The feeding of diets with the supplementation of 0.1% Lacto Sacc and 0.1% Yea Sacc of broiler chickens increased significantly triiodothyronine level in the blood plasma from 1.27 to 1.90 and 1.92 nmol/l .

Figure 3 showed that the level of thyroid stimulating hormone in the blood plasma was not significantly different in the supllementation of 0.01 % Avotan, 0.1 % Lacto Sacc and 0.1 % Yea Sacc in the diets of broiler chickens.

This study showed that the level of thyroxine in the blood plasma did not change significantly in the supplementation of Avotan, Lacto Sacc and Yea Sacc in the diets. Plasma level of triiodothyronine however increased in the supplementation of Lacto Sacc and Yea Sacc in the diets of broiler chickens. The results for the nutritive antibiotic confirmed our previous findings that the level of thyroxine in the blood plasma did not change with the supplementation of payzone, chlortetracycline and virginiamycine, but tendency was noticed in this study for an increase of plasma level of triiodothyronine with the supplementation of these antibiotics in the diets of broiler chickens (Chotinsky, 1999). Yen et al. (1985) also demonstrated that in pigs the weight of thyroid gland and the plasma concentration o L-3,3,5-triiodothyronine and thyroxine were not altered by carbadox. According to Davison and Freeman (1983) thyroid function was not affected after feeding aureomycin , but penicillin fed chickens had a greater plasma growth hormone concentration than the control birds. However, other authors reported that amprolium and payzone significantly decreased thyroid activity (Anderson and Gary- Anderson, 1973).

The present study reports for the first time the influence of probiotics on the level of thyroid hormone in the blood plasma of broiler chickens and provides new interesting data about a possible causal relationship between the growth promoting ef-

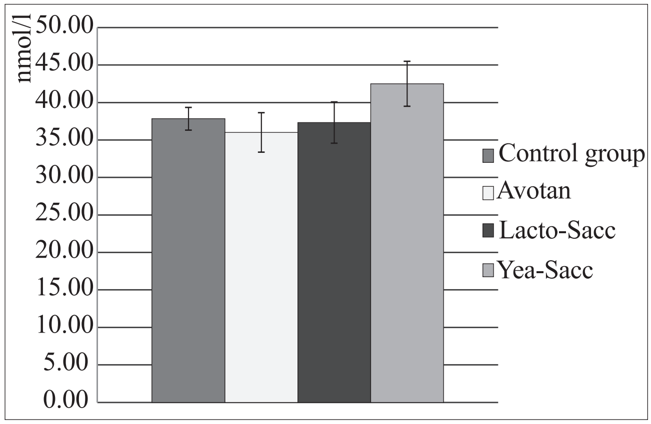


**Fig. 1. The level of thyroxine in the blood plasma of broiler chickens (P < 0.05)**

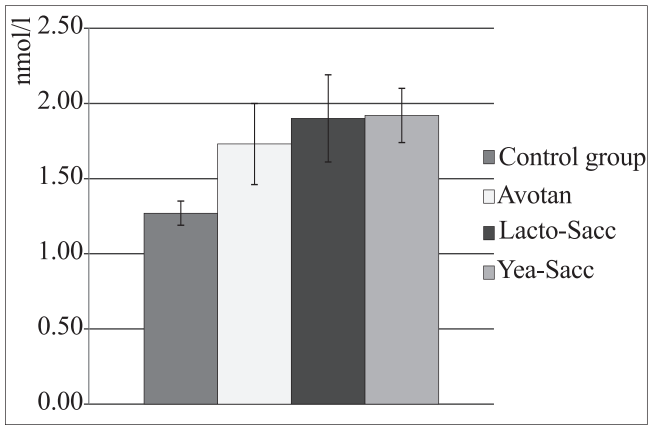


**Fig. 2. The level of triiodothyronine in the blood plasma of broiler chickens *P<0.05**

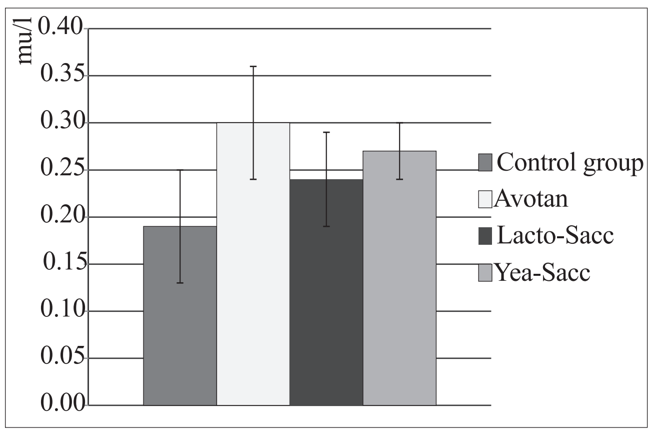


**Fig. 3. The level of thyrothrofic hormone in the blood plasma of broiler chickens**

fect of probiotics and thyroid hormone. Similar changes in the level of thyroxine and triiodothyronine in the blood plasma have been recorded in developing germfree rats as compared to the conventional rats (Uuai and Mitsuma, 1978). In the germfree rats, the rate of hepatic conversion of thyroxine into triiodothyronine was higher in the liver than that of the conventional rats and decreased from the bile salts (Ukai and Mitsuma, 1981).

Some authors have considered Probiotics as an alternative to antibiotics growth promotors, their competitive exclusion of intestinal indigenous microflora, to stimulate a favorable microbial balance, to diverse metabolic activity, to modified breakdown the bile acids and their interaction with the mucosal immune system (Panda et al., 2000; Ounwehand and Salmrnen, 2002; Teitelbaum and Walker, 2002; Chotinsky et al., 2003; Simon et al., 2003).

Usually bile acids are secreted in conjugated form and are deconjugated by bacteria in the ileum and large intestine. The majority of bacteria capable of deconjugating bile acids are anaerobes. *Lactobacillus* species found in the intestinal tract varied in the ability to conjugated bile acids. Most species of *Lactobacillus* isolated from human feces deconjugated sodium taurocholate and glycocholate (Gilliland and Speck, 1977). In the presence of Lactobacillus increased the concentration of the nonconjugated bile salts and the hydrolytic activity in the intestinal content (Tannock et al., 1989, 1994). It was supposed that the depretion in the growth of the conventional chickens depended on the deconjugation of bile salts from *Streptococcus faecium* attached to the duodenal epithelial cells (Fuller et al., 1983; Cole and Fuller, 1984). The antibiotics depressed *S. faecium* in the digestive tract and in this way lowered the deconjugation of the bile salts and bacterial hydrolase enzymes. Cole and Fuller (1984) found that Clostridium perfringens, streptococci and some of the bifidobacteria and lactobacilli were able to deconjugate all substrates, whereas the bacteroides deconjugated only the taurine conjugates bile acids and the coliform were completely inactive. Subtherapeutic levels of avoparcin, bacitracin, efrotomycin, lincomycin, penicillin G procaine and virginiamycin decreased cholytaurine hydrolase activity in ileal homogenates, which was the first step in bile acid transformation (Feighner and Dashkevicz, 1987).

It was found also that the colonization of the gut with Lactobacilli stimulated immune system (Famularo et al., 1997). They were able to cross the intestinal lumen into the spleen and other organs where they stimulated phagocytic activity (Deitch et al., 1990). *Lactobacillus* stimulated various aspects of the immune system; including phagocytes function of macrophages, natural killer cells, monocytes and neutrophils (Drisko and Giles, 2003). Tortuero et al. (1995) showed that the phagocytes activity in the cells and the concentration of interleukin-2 increase in ileum in piglets treated with *Streptococcus faecium* M-74 and *L. casei* spp. Rats colonized with *L. plantarum* + *E. coli* had significantly higher total IgA levels and marginally higher IgM and IgA antibody levels against *E. coli* than those colonized with *E. coli* alone (Herias et al., 1999).

Probiotics have also been found to increase inflammatory cytokines, such as interleukins (Drisko and Giles, 2003). Cytokines are large (8-60 kDa soluble polypeptide mediators that regulate growth differentiation and function of many different cells types. They are released fro the immune cells and involved in the regulation of immune processes (Tannock, 1997; Macfarlane and Cummings, 1999). Cytokines are not only restricted to cells of immune system, but are also found in many others tissues including enterocytes, endocrine glands, brains etc.(Turnbull and River, 1999 ).The intestinal mucosa is one of the biggest immune organs of the body and all the types of immunocompetent cells are identified (Friedman et al., 2003). Small intestine intraepithelial lymphocytes (IELs) comprise complex population of T cells, which are part of the gut associated lymphoid tissues (Klein, 1996). The investigation of Wang (1996) indicates that hormones of the hypothalamus-pituitary- thyroid axis exert either positive or negative regulatory effect on intestinal intraepithelial lymphocytes (IELs) depending upon the particular hormone.

Cytokines are produced in the thyroid gland by intrathyroidal inflammatory cells, in particular lymphocytes, as well as by the thyroid follicular cells (TFC) and may enhance the autoimmune process (Ajjian et al., 1996) . These authors noticed that they could also modulate both growth and the function of TFC.

Probiotic organisms may interact with the immune system at many levels, including cytokines production, macrophage phagocytosis, mononuclear cell proliferation etc. (Famularo et al., 1997; Schiffrin et al., 1997). *In vitro* bifidobacteria induce formation of large amounts of IgA (Yasui and Ohwaki, 1991).

## Conclusion

The study demonstrates that supplementation of Lacto Sacc and Yea Sacc in the diet increase the level of thyroid hormones. Probably this is associated with alteration in pool of bile acids and of the immune response, in particular of cytokines expression. Further studies are necessary to improve understanding potential implication of cytokines.

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