DRF-300
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research

Current Trends with Enzymes Applications in Industrial Broilers Production

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10.18805/ajdfr.DRF-300

ABSTRACT

Background: This study was conducted to analyse the effect of different enzymes and selenium on production performance and slaughter parameters of Ross (308) broilers production.

Method: There were four groups in this experiment, two of them were control and the two test groups. All groups had the same number of one-day-old chickens (20250), moved in the same day. 100 chickens were selected randomly for future analyses. The chickens in the control group were fed with the standard commercial diet which contained crude protein (22.04%, 18.55%, 17.89%) and metabolic energy including selenium (11.99 MJ/kg, 12.64 MJ/kg, 12.79 MJ/kg) for starter, grower and finisher phases respectively. The basal diet was supplemented with: HiPhos - 6 phytase, WX - endo-1,4- betaxylanase, VP-endo-1,3(4)- beta-glucanase and ProAct - serine protease. The experimental group also used organic selenium.

Results: Production performance and carcass parameters were not statistically different among treatment groups. Only mortality was decreased in one of the experimental groups ($p<0.004$). Whereas, the use of enzymes and selenium did no effect on the production performance and carcass parameters. The results indicate reduced feed cost of broilers diet with the use of enzymes as feed components. Result from presented work can be considered as complementary contribution in in broiler production.

Key words: Enzymes, Feed cost, Production parameters, Ross broilers, Selenium.

INTRODUCTION

In recent years, the poultry industry has been trying to improve its production by adding different feed additives to broilers diets. The main goal of the poultry industry is to reduce the price of concentrated feed. In the market, different producers supply different types of enzyme and their implementation depends on production, equipment, price and raw materials.

The use of enzymes in poultry nutrition is of great importance as they improve nutrient digestibility and utilization (Fry *et al.,* 1957). Obtained results in the last few years have shown that the inclusion of different enzymes in poultry feed improves utilization of more nutrients, especially if meals contain a higher percentage of non-starch polysaccharides (Smiljaniæ *et al.,* 2007; Van Emmenes *et al*., 2018; Woyengo *et al.,* 2019). Enzyme addition to corn, wheat, barley and rye-based diets significantly improve body weight gain (BWG) and feed conversion ratio (FCR) in broilers (Bedford and Schulze, 1998; Cowieson *et al.,* 2006; Ingelmann *et al.,* 2018). Generally, enzymes work in two steps, described as the an ileal phase and a cecal phase (Bedford 2000; Amerah *et al.,* 2017). During the ileal phase, enzymes remove fermentable substrates. During the cecal phase, they degrade products of sugars, such as xylose and xylo-oligomers, then sugars are fermented by cecal bacteria, which can stimulate the production of volatile fatty acids (VFA) and the growth of specific beneficial bacteria (Lee *et al.,* 2017).

Enzymes produce manno-, galacto-, gluco-, or xylo-oligomers, which are similar to prebiotics and facilitate the proliferation of health-promoting bacteria such as Lactobacillus and Bifidobacterium, during the process of depolymerization of various polysaccharides in the diet (Monsan *et al.,* 2007). On the other hand, xylanase may increase the access of endogenous and exogenous enzymes of protein and starch within the endosperm cell (Cowieson *et al.,* 2006) in a corn-based poultry diet by breaking down the highly branched insoluble arabinoxylans in the cell wall.

Protease enhanced degradation of soybean meal protein in the gut notably and the mode of action of protease are wholly allied with the digestibility (Woyengo *et al.,* 2019). Phytase can enhance the utilization of energy, protein and minerals, suggesting that higher amounts of alternative feedstuff could be used in the presence of enzymes (Bedford and Schulze 1998; Bedford 2000; Cowieson *et al.,* 2006; Monsan *et al.,* 2007; Yang *et al.,* 2010; Amerah *et al.,* 2017; Lee *et al.,* 2017; Ingelmann *et al.,* 2018; Woyengo *et al.,* 2019). Corn-based diets supplemented with multiple types

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How to cite this article: Bilić-Šobot, D. (2023). Current Trends with Enzymes Applications in Industrial Broilers Production. Asian Journal of Dairy and Food Research.doi:10.18805/ajdfr.DRF-300.

Submitted: 03-12-2022 **Accepted:** 22-05-2023 **Online:** 07-06-2023

of enzymes significantly improved the growth performance, gut health and FCR of broilers (Khan *et al.,* 2006; Abudabos 2010; Amerah *et al.,* 2017).

In this study, the source of Selenium (Se) was investigated and has been recognized as an important dietary nutrient that improve the broilers health and productivity. About 60 years ago, scientists realized that Se is important for human and animal health because it plays a role in the immune function and the thyroid gland. Se is an important supplement for broiler concentrated diets and is essential for growth (Zia Waseem *et al*. 2018; Yoon *et al*. 2007; Wang and Xu 2008) immune competence (Cai *et al.,* 2012), antioxidant (Peng *et al.,* 2007; Wang *et al.,* 2011) reproductive functions and immunocompetence (Leeson *et al.,* 2008). Se has an essential role in major metabolic pathways including antioxidant defence systems (Surai *et al.,* 2017), immunity (Arthur *et al.,* 2003) and thyroid hormone metabolism (Brown and Arthur, 2001). The primary source of Se for humans is food. This study was designed to analyze the effect of two different sources of Se used in broilers diets and its presence in broilers meat. Considering that two different manufacturers are analysing, the assumption is that one of them will influence production parameters and increased presence of Se content in the final product-meat.

MATERIALS AND METHODS

Location, chickens and housing

The experiment was carried out at commercial family farms in Pivka, Slovenia, which cooperated with food company Pivka Perutninarstvo d d, Slovenia, in the 2020 year. For this trial four broilers farm facilities were selected, two of them were selected as experimental groups and two were as control groups. One-day-old hybrids of Ross were obtained from commercial hatchers of fooder production company Jata Emona, Slovenia. Farm facilities No. 5 and 6 were experimental groups (with the addition of DSM enzymes, organic Se Selsaf®), while farm facilities No. 4 and 7 were control groups (without enzyme addition and with and Se SelPlex®). Since this experiment was performed for commercial use of the company Pivka Perutninarstvo d. d. in Slovenia, the test included a standard number (20250) of immigrated one-day-old Ross chicken for their production. At the end of the experiment, 100 chickens were selected randomly for future analyses. In farm facilities, lighting and temperature were controlled according to the standard procedure recommended for Ross (308) broilers (Aviagen, 2018). The broilers were raised until they reach 40 days of age, with an average live weight of 2.20-2.50 kilograms.

Formulation of diets, used enzymes and selenium in broilers feed

For the control facilities, the standard feed was used for both groups of broilers. Basal diets contained corn ingredient (44.0%, 41.0% and 43.0%), wheat (8.0%, 20.0% and 20.0%), soybean meal (36.0%, 29.0% and 29.0%), crude proteins (22.04%, 18.55% and 17.89%) and metabolic energy (11.99 MJ/kg, 12.64 MJ/kg and 12.79 MJ/kg) (Table 1). Control diets contained premix with added Se Sel-Plex®, selenised yeast, [an organic source derived from Saccharomyces cerevisiae from the company Alltech Alltech, 2021)] and inorganic Se in the form of sodium selenite.

In the experimental facilities, the enzymes used as a feed additive (involved in premix) were: Ronozyme® HiPhos - 6 phytases, Ronozyme® WX - endo-1,4- betaxylanase, Ronozyme® VP - endo-1,3(4)- beta-glucanase and Ronozyme® ProAct - serine protease. Also, these groups had added organic selenium Selsaf® in the premix (Table 2). Enzymes and Se used in the experimental group were obtained from the same producer, DSM Nutritional Products (see: Agrovit, 2021). Selsaf® is a natural source of selenomethionine and selenocysteine provide double protection against both oxidative and pathogenic stress not only for the animal but also exhibiting benefits for farmers and consumers by sustaining animal health and enhancing zootechnical performance.

Experimental and control diets were formulated with additions of sunflower and multi enzymes to meet or exceed the breeder's recommendations (Aviagen, 2018). The starter diet was in the form of crushed pellets, while the grower and finisher diets were in the form of pellets.

Analysis of selen in broilers feed and meat

Samples of experimental diets were taken in triplicates on the day when concentrated feed was produced. Therefore, samples of chicken breast were also taken in triplicates at the slaughter line. All products of food company Pivka Perutninarstvo d. d. have certificate of the "High-quality of chicken meat" which must gratify minim requirement of Se for broilers concentrated feed from 0.3-0.5 mg/kg and 8.25 µg/100 g for the chicken meat. All samples were sent to the accredited laboratory of the Institute of Food Safety, Feed and Environment at Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia for future analyses. The feed was analysed using the in-house method, elements in the feed was determined with inductively coupled plasma mass spectrometry ICP-MS according to the methods descibed in AOAC (2000) with the standard operative procedure. The concentration of feed additives was in accordance with Regulation EU (2003). At the end of the experiment, meat samples were taken on cutting line, vacuum-packaged and delivered to the laboratory on the same day. For these analyses, inductively coupled plasma emission spectrometry was used, (ICP-MS spectrometry), as described by Tanner *et al*. (2002).

Carcass measurements, production and economically data of experiment

Samples of carcass, thigh, shorter, back, wings, spikes and breast were taken from facility No. 4, 5, 6 and 7. For measurement of carcass quality, 100 chickens were taken

from each facility. Also, during the breeding, body weight gain, feed conversion ratio, mortality and feed cost were measured. Production parameters were calculated in kilograms and percentage. At the end of the trial, cost of concentrated feed using current prices of raw material was calculated. The prices were calculated according to the optimizing program "WinMix" soft, which was also used for nutrient calculation of meals. The prices were calculated per 1000 kg/€.

Statistical procedure

Data were analysed using a one-way analysis of variance (effect of the experimental group). All statistical analyses were performed using the statistical software SPSS (IB SPSS21). Results were presented as mean±standard deviation. Where significant treatment effects were observed, differences between treatment means were compared using the Tukey test. Values of $p<0.05$ were considered statistically significant.

Table 1: Ingredient composition of the control diets (as fed) for 0-15, 16-35 and 35-40 of age.

Ingredients in diet (kg/ton)		Ross 308 Starter 0-15 d[1]	Ross 308 d grower 16-35	Ross 308 finisher >35 d
	Cost/ton	279.51	251.00	248.00
Wheat	170.00	80.00	200.00	200.00
Yellow maize 7.5%	175.00	440.00	409.40	429.20
Soya O/C 46%	320.00	360.00	290.00	290.00
Sunflower O/C 34 %	248.00	20.00	/	/
Soy oil	605.00	33.00	35.00	35.20
Corn gluten 60%	800.00	20.00	/	/
Limestone 37%	30.00	16.00	14.00	14.00
Monocalcium phos	482.00	16.00	15.00	15.00
Salt	100.00	2.10	2.10	2.10
Sodium bicarbonate	300.00	1.50	1.50	1.50
Alimet (MHA)	1600.00	3.00	3.00	3.00
L Threonine in px	/	0.70	/	/
Lysine HCL in px	/	/	/	/
PRAH - BFK[2]		/	20.00	/
Se selplex in px		0.20	0.20	0.20
Premix[3]	/	10.00	10.00	10.00
TOTAL		1000.00	1000.00	1000.00
Calculated values of diet				
ME poultry	MJ/kg	11.99	12.64	12.79
Crude protein	g/kg	220.42	185.55	178.93
Dig arginine P	g/kg	15.44	12.77	12.27
Dig isoleucine P	g/kg	9.87	8.24	7.95
Dig lysine P	g/kg	14.31	10.99	9.94
Dig Meth+Cyst P	g/kg	9.183	8.05	7.32
Dig methionine P	g/kg	4.57	3.95	3.34
Dig threonine P	g/kg	9.53	7.45	7.18
Dig tryptophan P	g/kg	2.49	2.35	2.23
Dig valine P	g/kg	10.84	9.23	7.79
Avl phosphorus P	g/kg	5.24	4.41	4.04
Calcium	g/kg	11.03	9.86	9.06
Chloride	g/kg	1.84	1.83	1.55
Potassium	g/kg	10.24	8.56	8.23
Sodium	g/kg	1.51	1.47	1.33
Fat	g/kg	54.42	67.19	70.73
Fibre	g/kg	34.00	31.33	30.88

Explanation: d1- day; PRAH - BFK2 - recycled row material; Premix3 - Vitamin premix/kg contained for grower diet: Vitamin A (retinal acetate) 1.000.000 I.U., D3 (cholecalciferol) 400.000 I.U., E (tocophery acetate) 10.000 I. U., K3 (sodium bisulphide) 700 mg, B1 300 mg, B2 600 mg, Niacin 5.000 mg, Pantothenic acid 1800 mg, B6 400 mg, Folic acid (B9) 200 mg, Biotin 15 mg, B12 1.500 mg, Choline chloride 66.000 mg. Mineral premix/kg contained: Mn 31.3 g, Fe 21.9 g, Zn 22.5 g, Cu 8 g, Kl 0.18 g, Na selenite 0.033, organic Se 10 g.

RESULTS AND DISCUSSION

Table 3 shows the production performance of broilers fed diet supplemented with DSM enzymes and selenium. In the treated group, body weight, live weight, average daily gain, feed conversion and European production efficiency factor (EPEF) were not statistically different. The feed conversion ratio control facility No. 4 was lower than that of the experimental facility No. 5. Average daily gain (ADG) was slightly lower in control facilities No. 7 than that of the other facilities, especially in experimental facilities No. 5. Mortality

Table 2: Ingredient composition of the experimental diets (as fed) for 0-15, 16-35 and 35-40 of age.

Ingredients in diet (kg/ton)		Ross 308 Starter 0-15 d[1]	Ross 308 d grower 16-35	Ross 308 finisher >35 d
	Cost/ton	268.45	250.00	240.00
Wheat	170.00	102.00	200.00	200.00
Yellow maize 7.5%	175.00	490.00	411.00	456.30
Soya O/C 46%	320.00	308.00	260.00	230.00
Sunflower O/C 34 %	248.00	20.00	40.00	50.00
Soy oil	605.00	15.00	30.00	30.00
Corn gluten 60%	800.00	20.00	/	/
Limestone 37%	30.00	13.00	11.00	10.50
Monocalcium phos	482.00	9.50	10.00	7.00
Salt	100.00	2.10	2.10	2.10
Sodium bicarbonate	300.00	1.50	1.50	1.50
Alimet (MHA)	1600.00	4.60	4.00	3.00
L threonine in px	1400.00	0.93	0.73	0.52
Lysine HCL in px	/	3.41	2.91	2.73
PRAH - BFK[2]	/	/	16.60	/
Se Selsaf in px		0.20	0.20	0.20
Premix[3]	/	10	10	10
Ronozyme proAct in px	/	0.20	0.20	0.20
Ronozyme VP in px	/	0.20	0.20	0.20
Ronozyme WX 2000 in px	/	0.10	0.10	0.10
Ronozyme HiPhos 10.000 in px	/	0.20	0.20	0.20
Total		1000.00	1000.00	1000.00
Calculated values of diet				
ME pultry	MJ/kg	12.55	12.97	13.39
Crude protein	g/kg	226.55	205.32	185.47
Dig arginine P	g/kg	13.08	12.12	10.76
Dig isoleucine P	g/kg	8.53	7.65	6.80
Dig lysine P	g/kg	12.50	11.20	9.80
Dig Meth+Cyst P	g/kg	9.30	8.50	7.60
Dig methionine P	g/kg	5.92	5.41	4.73
Dig threonine P	g/kg	8.40	7.50	6.60
Dig tryptophan P	g/kg	2.18	2.01	1.77
Dig valine P	g/kg	9.40	8.50	7.67
Avl phosphorus P	g/kg	4.80	4.20	3.90
Calcium	g/kg	9.60	8.40	7.80
Chloride	g/kg	2.30	2.26	2.25
Potassium	g/kg	8.82	8.39	7.49
Sodium	g/kg	1.60	1.60	1.60
Fat	g/kg	39.64	54.87	64.81
Fibre	g/kg	37.44	38.87	38.13

Explanation: d1 -day; PRAH - BFK2 - recycled row material; Premix3 (px) - Vitamin premix/kg contained for grower diet: Vitamin A (retinal acetate) 1.000.000 I.U., D3 (cholecalciferol) 400.000 I.U., E (tocophery acetate) 10.000 I. U., K3 (sodium bisulphide) 700 mg, B1 300 mg, B2 600 mg, Niacin 5.000 mg, Pantothenic acid 1800 mg, B6 400 mg, Folic acid (B9) 200 mg, Biotin 15 mg, B12 1.500 mg, Choline chloride 66.000 mg. Mineral premix/kg contained: Mn 31.3 g, Fe 21.9 g, Zn 22.5 g, Cu 8 g, Kl 0.18 g, Na selenite 0.033, organic Se 10 g.

was recorded on daily basis and was lower in experimental groups.

Table 4 shows the effect of diet supplemented with DSM enzymes and Se on carcass parameters of treated broilers. The addition of phytase, betaxylanase, beta-glucanase, serine protease and both Se (organic and inorganic) did not effect on dressing, thigh, drumstick, back, wing, spike and breast. Similar results were reported by Van Emmenes *et al*. (2018) where carcass and cutting parameters were not affected by phytase.

Table 5 shows the effect of Se content on feed and meat. The concentrated meal, Bro-finisher-final showed the highest concentration of Se content (DSM). The results indicated that Se is increased in experimental groups for chickens meat - which satisfies the parameters related to the higher quality certificate related to selenium. Especially if we compare the results of experimental groups (objects 5 and 6) and the control group (objects 4 and 7).

Table 6 presents a decreased price of produced feed with the addition of enzymes. Especially the reduction was observed in the starter and finisher concentrated meals where the final cost of the standard meal decreased from 11.06 to 8 euro per 1000 t. Higher production parameters during the whole rearing period were considered profitable (the ideal number for the Pivka Perutninarstvo d. d. is about 400).

This trial investigated the effect of multienzymes, organic and inorganic Se addition on the production performance of commercial broiler. Enzymes have been used as trends and anti-nutritional factors to increase feed utilization and to improve performance traits, as well as to decrease stress situations (Jia *et al.,* 2008; Attia *et al.,* 2012;

Table 3: Results of production parameters of broilers supplemented with DSM enzymes and Se (LS Means±SD).

Facilities	N	Facilities no. 4 (control group)	Facilities no. 7 (control group)	Facilities no. 5 (experimental group)	Facilities no. 6 (experimental group)	P-values
Bodyweight day 0 (kg)	100	222.60±233.75	199.85±205.26	226,80±234.22	199.95±205.28	0.966
Bodyweight day 14 (kg)	100	235.15±241.34	229.75±238.35	244.95±251.48	235.2 ±241.46	0.998
Bodyweight day 27 (kg)	100	220.95±228.01	280.6±290.93	250.30±260.24	250.60±265.98	0.915
Live weight (kg)	100	13.25±29.67	19.10 0±21.64	16.80 ±20.51	13.40 ±15.01	0.809
Average daily gain (kg)	100	262.55±333.95	164.40±250.31	293.90±358.75	243.55±351.74	0.636
Feed conversion (kg)	100	314.95±324.45	334.10±345.44	326.35±336.96	302.35 ± 311.17	0.990
EPEF*	100	233.05±316.82	333.35±407.71	237.8 ±348.05	249.95±340.16	0.786
Mortality %	20250ð	511.44±204.76[a]	679.03±220.36 [b]	505.77±182.95[a]	566.46 ± 206.24 [a]	0.004

Explanation: N -number of measured broilers, EPEF*- European production efficiency factor /Viability (%) × BW (kg)/Age (day) × FC (kg feed/kg gain)/ 20250*- number of chicks pre each facilities.

Table 4: Results of carcass parameters of broilers supplemented with DSM enzymes and Se (LS Means±SD).

Facilities	N	Facilities 4-control group	Facilities 7-(control group)	Facilities 5-(experimental group)	Facilities 6-(experimental group)	P-values
Dressing (%)	100	54.13 ±26.84	58.75 ±26.73	57.81 ±32.65	53.06 ±28.14	0.915
Thigh (kg)	100	108.60±117.66	119.65±127.04	108.30±111.62	101.10±103.82	0.965
Drumstick (kg)	100	124.75±128.67	97.65±101.02	99.25±102.11	92.15 ±94.94	0.777
Back (kg)	100	162.45 ±167.73	138.30±142.30	138.90±148.12	133.20±139.66	0.928
Wing (kg)	100	82.75 ±84.98	73.50 ±75.65	71.50 ±73.57	65.65 ±67.56	0.913
Spike (kg)	100	9.00 ±9.34	8.60 ±9.07	8.60 ±9.27	7.30 ±8.45	0.939
Breast (kg)	100	381.00 ±391.64	331.95±342.22	317.35±326.51	302.15±313.62	0.897

Table 5: Results of selenium content in feed and meat.

Feed	Se content (mg/kg)
Bro-starter	0,392
Bro-finisher	0,435
Bro-finisher-final	0,443
Meat	Se content (µg/100 g mesa)
Chicken breast, 4- (control group)	13,3
Chicken breast, 7- (control group)	16,8
Chicken breast, 6- (experimental group)	20,2
Chicken breast, 5- (experimental group)	20,4

Table 6: Comparison of prices of standard and experimental feed with the addition of enzymes and Selenium.

Standard feed	Price (€/t)	Experimental feed	Price (€/t)	The difference in price (€/t)
Bro-starter	279,51	Bro-starter	268,45	11,06
Bro-finisher (grower)	251	Bro-finisher	250	1
Bro-finisher-final	248	Bro-finisher-final	240	8
Production number*	Facility No. 4 (control group)	Facility No. 7 (control group)	Facility No. 5 (Experimental groups)	Facility No. 6 (Experimental groups)
	367,58	369,81	370,38	340,65

Production number* - calculated on whole data received from a farmer and internal protocol of the company Pivka Perutninarstvo d. d. (consumption of food, transportation, mortality, veterinary drugs and services).

Al-Harthi *et al.,* 2018). Poultry industries are trying to decrease the feed costs of concentrated meals without diminishing their quality. The Company Pivka Perutninarstvo d. d. monthly produce 3 350 000 kg of concentrated meals for broilers, parent flocks and laying hens for their subcontractors. Any trends that can economically improve production is welcome.

Autor's Meel *et al.* (2021) and Amerah *et al*. (2017) indicated that hyalase, protease and amylase enzymes (multienzymes) increased ADG and FCR in Ross (308) broilers. Other production performances and carcass parameters were not affected by the addition of enzymes. Similar results were obtained by Goodarzi Boroojeni *et al*. (2017), who reported that Ronozyme ProAct and VP-Protease improved FCR, but decreased feed intake compared to the control group. In contrast, Olkowski *et al*. (2010) reported that Ronozyme VP (endo-1,3 (4)- β-glucanase) with the activity of pentozanase, hemicellulose and pectinase decreased FCR and live weight compared to the control group.

The results of the present study indicated no visible effects of multienzymes and Se on carcass characteristics, which agrees with previously published studies (Liu *et al.,* 2015; Ozturk 2017; Hossein Zadeh *et al.,* 2018). Results of the present study also showed that the addition of Se of different producers had no effects on the growth performance, which is in agreement with previous studies (Yoon *et al.,* 2007; Oliveira *et al.,* 2014; Hossein *et al*. 2018; Marco *et al.,* 2021). The present study showed a high concentration of Se in chicken breast. In agreement with the current results, Woods *et al*. (2020) showed that Se had no effect on the live weight of broilers but reported an increased concentration of Se in chicken breast. Available published data showed no effect of Se on production and carcass characteristics; this might be since Se had no effect on production performances, especially when broilers were reared under normal commercial conditions.

In this experiment, DMS products proved to be more effective for parameters of High-quality certificates and impacts on the economy of feed costs. Similar results were obtained by Marco *et al*. (2021), where the addition of selenised yeast in broilers feed (0.465 mg/kg) was increased in comparison with elemental and sodium sources of Se. In our research, an organic form of selenium Selsaf from DSM producer, (saccharomyces cerevisiae) showed better results compared to the control group which had Sel-Plex Se from Alltech company. This trial assured that DSM products obtained the same or better results with decreased cost of concentrated meal/1t, especially for starter and finisher feed (Table 6).

CONCLUSIONS

The use of enzymes as an everyday trend in broilers productions, are essential for production parameters and economical justification. The present study indicated that the addition of enzyme and selenium of different companies had no effects on the growth performance and carcass characteristics of Ross broilers hybrids. On the other hand, DSM enzymes decreased the feed cost and improved the content of selenium in concentrated feed and meat of broilers. A few years ago, the use of chicken meat in human nutrition increased. Industries with a rounded production cycle (hatchery station, feed factory, rearing of one-day-old chickens, slaughterhouse, production of fresh meat) are forced to look for alternative methods to increase the quality and quantity of chicken meat while reducing the number of days of raising chickens with the use of various additives in chicken nutrition. The presented study is a good indicator of the use of enzymes in the nutrition of chickens to increase the amount of produced meat, with a reduction in the cost of produced animal feed.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Pivka's Perutinarstvo staff for their cooperation and efforts during the conduct of this experiment.

Conflict of interests

The authors declare no conflict of interest.

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