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Research Article
Inclusion of *Secale cereale* and Pentosanases in the Ration of Laying Hens: Exploring its Effect on Egg Production and Concentrate Intake and Searching the Optimal Combination in a Diet

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Abstract

Background and Objective: Antinutritional factors are usually found in cereals, these correspond to molecular complexes structured by carbohydrates present in the grains, which increase the viscosity of the food bolus, resulting in diminished organic matter digestibility, food intake and egg production. These polymers are known as soluble non-starch polysaccharides (NSPs). The objective of this experiment was to assess the effect of incorporating *Secale cereale* (SEc) and pentosanases (EEp) in the diets of laying hens (Lohmann LSL hybrid) on their egg production (EP) and concentrate consumption (CC), optimal combination between the main factors was estimated for EP and CC. **Materials and Methods:** The trial was carried out on a commercial egg-producing poultry farm (Valparaíso Region, Chile). A completely randomized experimental design was used, with a 4×5 factorial arrangement of two factors (four levels for SEc and five levels for EEp). Ten repetitions were used per treatment, where each experimental unit corresponded to one cage with five hens. A contour curvature analysis was carried out to determine the optimal level of inclusion of SEc with EEp. The experimental period lasted 84 days. **Results:** There was an interaction effect of SEc and EEp on EP and CC. The optimum level of inclusion for EP was 200 g kg^{-1} SEc and 1 g kg^{-1} EEp, with average production of 396 units, for CC, the optimum value observed corresponded to 200 g kg^{-1} SEc with 0.5 g kg^{-1} EEp (47.56 kg). The lowest EP and CC responses, regardless of the EEp level in the diet, was observed with the inclusion of 600 g kg^{-1} SEc. The contour area curvature analysis for EP gave a high response curve zone between 130-390 g kg^{-1} SEc and 0.38-1.57 g kg^{-1} EEp, the highest response levels for CC were 120-350 g kg^{-1} SEc and 0.43-1.75 g kg^{-1} EEp. **Conclusion:** In this research it was possible to establish that high level of SEc inclusion in the diet affects negatively eggs production and concentrate intake. However, when pentosanases are included in the diet improve these variables. These results suggest possible antinutritional factors of SEc that could have caused negative effects on the hens' physiological response.

Key words: Concentrate consumption, hen production, pentosanases, rye antinutritional factors, poultry production, egg production

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Some cereals contain antinutritional factors associated with molecular complexes structured by certain carbohydrates present in the grains, resulting in diminished digestibility of the organic matter in the gastrointestinal tract of fowls. These are known as soluble non-starch polysaccharides (NSPs). They are normally found in important quantities in rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), which are frequently used as ingredients in animal feed formulations[1,2]. These anti nutritional factors alter the bioavailability of nutrients in the intestine, thus altering nutrient absorption processes and reducing the efficiency of food conversion and the contribution of metabolizable energy from the feed, this may in turn affect some of the fowls' production parameters[3].

Non-starch polysaccharides are carbohydrates which form part of the cell wall of cereals, consisting principally of β-glucans and pentosanes; the commonest form of the latter in plants is the molecular complex arabinoxylan. These polymers are observed in a complex spatial arrangement, in a matrix in which they interact physically and chemically with cellulolitic and non-cellulolitic polysaccharides, lignin and glycoproteins, found between the microfibrils of cellulose[4,5]. When dissolved in water, the majority of polysaccharides generate a specific viscosity in the solution; this is dependent, among other factors, on the size and molecular weight of the particles in solution, the degree of spatial ramification and linearity of the polymers, the presence of electrochemical charges in the molecular groups involved, the degree and type of interaction between the water molecules and the polysaccharides, and finally, their concentration[6,7]. As the concentration increases, the polysaccharide molecules interact with one another and the water, forming a mesh which increases the viscosity of the solution, this can lead to the formation of a gel if there is a sufficient level of interactions. Given their hydrophilic capacity and considering the ideal humidity, temperature and pH conditions which exists in fowls' digestive systems, NSPs are highly likely to form a gel in the food bolus as it passes through the gastro-intestinal tract. Indeed, the arabinoxylan complexes present in *Secale cereale* (SEc) increase the viscosity of the chyme in fowls, reducing the velocity with which the bolus passes through the tract and blocking endogenous digestive enzyme activity and the absorption of nutrients in the intestine. The consequence will be an impact on the voluntary consumption of feed, conversion efficiency and egg production[8-10].

Fowls do not have the endogenous capacity to produce the hydrolytic enzymes necessary to depolymerise and digest NSPs molecular complexes[7]. Hydrolytic enzymes (pentosanases) are found in nature which degrade these NSPs, they are synthesised by a series of microorganisms (bacteria and fungi) and can be found in various ecological niches. This battery of microbial enzymes allows the glucosidic bonds in NSPs to be hydrolysed effectively. The endo and exo-activity of these exogenous enzymes would produce oligosaccharides, trisaccharides, disaccharides and some monosaccharides, increasing the digestibility of the feed.

The object of this investigation was therefore to assess the effect of the inclusion of *Secale cereale* (SEc) and an exogenous enzyme extract rich in pentosanases (EEp) in the feed of laying hens on egg production (EP) and concentrate consumption (CC). The optimum levels of SEc and EEp as a function of the variables EP and CC will also be determined by contour curvature analysis.

MATERIALS AND METHODS

The 84-days trial was carried out in a commercial egg-production plant located in Hijuelas in the Valparaíso Region of Chile. The experimental units (EU) were cages containing five hens (genetic line (hybrid): Lohmann LSL White) each, all in their first laying season, with an age at the start of the study of 210 days. All laying hens were managed under bioethical principles for animal care and welfare throughout the experiment, according to Guide for the Care and Use of Agricultural Animals in Research and Teaching[11].

The SEc (variety Petkus) contained 110 g kg^{-1} moisture (AOAC[12]: Official method 934.01), 95 g kg^{-1} crude protein (N× 6.25) (AOAC[12]: official method 984.13), 26 g kg^{-1} fatty matter(AOAC[12]: official method 920.39), 15 g kg^{-1} ash (AOAC[12]: official method 942.05), 28 g kg^{-1} raw fibre (AOAC[12]: official method 962.09) and 2.73 Mcal kg^{-1} MS metabolizable energy (Francesch[13]). The EEp was a commercial preparation of natural origin, purified from a controlled fermentation of the fungus *Aspergillus niger* and designed to generate enzymes of the pentosanases group.

The treatments consisted of the factorial combination of four inclusion levels were tested for SEc (0, 200, 400 and 600 g kg^{-1}) and five for EEp (0.0, 0.5, 1.0, 1.5 and 2.0 g kg^{-1}), with 10 repetitions for each treatment. All the experimental diets were formulated on the basis of the same requirements, thus ensuring that they were isocaloric and isoproteic, as well as satisfying critical levels of other nutritional requirements (Table 1). Nutritional composition of all diets was: metabolizable energy, 2.8 Mcal kg^{-1}, crude protein, 170 g kg^{-1}, raw fibre, 50 g kg^{-1}, calcium, 35 g kg^{-1}, phosphorus, 4 g kg^{-1}, lysine 7.6 g kg^{-1}, methionine+cysteine,

Table 1: Feeds by proportion of *Secale cereale* (SEc), combined in the experiment with the different dosage of pentosanases (EEp)[1]

Feed	Diet 1[2] (g kg^{-1})	Diet 2 (g kg^{-1})	Diet 3 (g kg^{-1})	Diet 4 (g kg^{-1})
SEc	0.00	200.00	400.00	600.00
Argentinean maize	470.46	313.77	157.58	0.00
Wheat flour	85.60	44.93	3.25	0.00
Gluten meal	16.70	16.70	16.70	72.77
Fatty acids	20.90	20.90	20.90	20.90
Australian lupine	66.70	66.70	66.70	66.70
Argentinean groundnut pellets	66.70	66.70	66.70	66.70
Argentinean soya	97.47	94.85	92.75	16.75
Deactivated soya	66.70	66.70	66.70	46.20
Ground seashells	82.20	82.32	82.45	84.16
Methionine	1.00	0.97	0.93	0.44
Tricalcium phosphate	21.57	21.46	21.34	21.38
Salt	3.00	3.00	3.00	3.00
Laying minerals	0.50	0.50	0.50	0.50
Laying vitamins	0.50	0.50	0.50	0.50

[1]EEp, exogenous enzyme extract, Each diet was combined with proportions of 0, 0.5, 1.0, 1.5 and 2.0 g kg^{-1} pentosanases, [2]Nutritional contribution in all diets (isocaloric-isoproteic): Metabolizable Energy: 2.8 Mcal kg^{-1}, Crude protein: 170 g kg^{-1}, Raw fibre: 50 g kg^{-1}, Calcium: 35 g kg^{-1}, Phosphorus: 4 g kg^{-1}, Lysine: 7.6 g kg^{-1}, Methionine+cysteine: 6.6 g kg^{-1}, Tryptophan: 1.8 g kg^{-1}

6.6 g kg^{-1} and tryptophan, 1.8 g kg^{-1}. The different treatments were provided to the EU *ad libitum* in the form of an all-mash, with mean grinding range from 0.2-2.5 mm, allowing the hens easy access to the concentrate.

The hens were randomly selected from a batch of 10000 hens, received at day of age, which were kept in floor (cement) covered with shavings, inside a shed, disinfected and protected of the environment, whose density corresponded to 65 chicks/m^2. This shed had gas bells, which supplied enough heat to maintain the ambient temperature at an average of 28°C. A gas bell was occupied for 1000 chicks and gradually removed after 20 days, reaching an average temperature of 21°C (approximately) and with a relative air humidity of between 60 and 70%, at six weeks old. These birds were kept in this shed under the same conditions until fifteenth week of age. Subsequently, they were transferred to their final posture cages.

The random selection of the EU (1000 hens) began at 27 weeks of age, when were randomly housed in the experimental cages (5 hens/cage). The acceptance range, in terms of the live weight of the bird, was 1.65±50 g. In addition, only healthy birds with normal posture performance were considered. The EU were physically isolated by brass separators and interspersed every three empty cages, in such a way to accurately measure EP and CC for each EU.

Egg collection was carried out daily between 15:00 and 18:00 pm, taking into account that the hens laid their eggs preferably in the morning. The EP was expressed in units (U), which was the mean number of eggs produced per treatment combination during the trial. The CC was determined by the difference between the initial amount of feed provided in the feeding trays (2 kg EU^{-1}) and the total weight of feed left over after 24 hrs (morning of the following day). The mean consumption per treatment throughout the experiment was expressed in kg.

The enzymatic extract of commercial origin (EEp) was generated from a controlled fermentation of the fungus *Aspergillus niger*, using bioreactors under industrial production conditions. The basic composition of EEp was constituted by 600 g kg^{-1} of enzymes belonging to the pentosanase group and the remaining 400 g kg^{-1} by inert substances, using the typical industrial process of enzyme extraction and purification. It was prepared in powder form (988 g kg^{-1} DM), which was incorporated directly over the concentrate during preparation of the experimental diets.

The birds were exposed to 24 h light day^{-1} for the first three days of the fowls' lives. From the fourth day to the eighth week, the chicks were exposed to 13 hrs constant light day^{-1}, following the lighting programme for Lohmann LSL hens. From week 9-17 they received 12.30 h constant light day^{-1}. From the eighteenth week the programme returned to 13 hrs light/day, and at the same time the light programme to stimulate egg production was started. This light programme was started with an increase of one h of light/day/week until 21st week of age when the laying stage began. At 21st week, the total supply of light was 16 h day^{-1}. The lighting programme throughout the trial was followed strictly with a total of 16 h constant light day^{-1}, including natural and artificial light.

Statistics: A completely randomized design was used with a 4×5 factorial arrangement, using ten repetitions per treatment. The first factor was the percentage of inclusion of SEc in the diet; the second was the inclusion of EEp. The response variables were EP and CC.

The general statistical model for this experiment was:

$$Y_{ijk} = \mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+e_{ijk}$$

Where:

Y_{ijk} = Observed value (EP or CC)
μ = Generalized mean
α_i = Effect of the ith level of factor α (SEc)
β_j = Effect of the jth level of factor β (EEp)
$(\alpha\beta)_{ij}$ = Interaction between the ith level of factor α and the jth level of factor β
e_{ijk} = Experimental error

Prior to ANOVA, the data were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test).Values of $p \leq 0.05$ were considered statistically significant. The results were analysed using the software SYSTAT 11[14] and STATISTICA V. 6.0 Stat Soft[15]. A contour area curvature analysis was also carried out for both EP and CC, using the Spline Cubic Programme (STATISTICA V. 6.0 StatSoft[15]).

RESULTS

There were interaction effects between SEc and EEp on EP and CC ($p<0.000$). Table 2 and 3 show the mean values observed for all the treatments. EP presents a negative trend if SEc is increased without the enzyme. However, this trend was reversed when EEp was added to the feed but the change is not linear. For example, crossed values were observed for the lines 0.5, 1.5and 2 g kg^{-1} EEp when the inclusion of SEc in the feed is increased from 200-400 g kg^{-1}. This evidenced the presence of an interaction between SEc and EEp, affecting EP. The interaction effect can likewise be observed when the change in EP is examined under the change from 0 g kg^{-1} EEp and 0 g kg^{-1} SEc (363.6 U) to a value of 299.7 U with 200 g kg^{-1} SEc, the EP rises again to values of 385.4 and 396 U, respectively, for the combinations of 0.5 g kg^{-1}×200 g kg^{-1} and 1 g kg^{-1}×200 g kg^{-1} (EEp×SEc). The latter was the highest EP value recorded in the trial. For the combinations 1.5 g kg^{-1} with 200 and 400 g kg^{-1} SEc, although the proportion of EEp was increased, the EP fell to 376.2 and 369.8 U, respectively. The combinations of 0.5 g kg^{-1} EEp with 200 g kg^{-1} SEc and 2.0 g kg^{-1} with 200 g kg^{-1} SEc produced the second and third highest EP responses (385.4 and 384.6 U).

For CC, the optimum value was observed (47.56 kg) corresponded to 200 g kg^{-1} SEc with 0.5 g kg^{-1} EEp. The cells with 200 g kg^{-1} SEc and 1 g kg^{-1} EEp, and 200 g kg^{-1} SEc and 1.5 g kg^{-1} EEp, produced the second and third best responses for CC, with means of 47.39 and 47.30 kg, respectively

Table 2: Effect of the inclusion of *Secale cereale* (SEc) and pentosanases (EEp) in the feed of laying hens on egg production (EP)

	SEc							
	Diet inclusion (g kg^{-1})							
	0		200		400		600	
EEp (g kg^{-1})	EP	SEM[3] U[1]	EP	SEM U	EP	SEM U	EP	SEM U
0.0	363.6[2]	7.64	299.7	5.67	262.6	11.23	198.1	5.67
0.5	367.5	7.03	385.4	8.91	344.7	7.88	225.1	8.78
1.0	360.5	5.07	396.0	4.56	373.8	6.64	222.3	9.03
1.5	364.2	8.17	376.2	9.12	369.8	5.78	214.2	5.98
2.0	369.1	9.23	384.6	11.21	366.1	8.87	228.3	7.77

[1]U, mean EP per treatments combination expressed in units (eggs), [2]Significant interaction effect ($p<0.000$) was observed between the main factors on EP, [3]Standard error mean

Table 3: Effect of the inclusion of *Secale cereale* (SEc) and pentosanases (EEp) in the feed of laying hens on concentrate consumption (CC)

	SEc							
	Diet inclusion (g kg^{-1})							
	0		200		400		600	
EEp (g kg^{-1})	CC[1]	SEM[3] kg	CC	SEM kg	CC	SEM kg	CC	SEM kg
0.0	44.48[2]	1.77	39.61	2.33	36.35	2.34	29.15	2.87
0.5	47.04	2.54	47.56	3.56	42.61	1.29	31.96	1.22
1.0	46.17	2.33	47.39	2.28	44.80	3.45	32.49	3.72
1.5	46.45	3.45	47.30	5.46	42.39	2.09	30.38	1.43
2.0	47.21	1.98	46.10	4.33	41.32	1.89	31.26	2.31

[1]CC, mean consumption per treatments combination during the whole experiment, [2]Significant interaction effect ($p<0.000$) was observed between the main factors on CC, [3]Standard error of mean

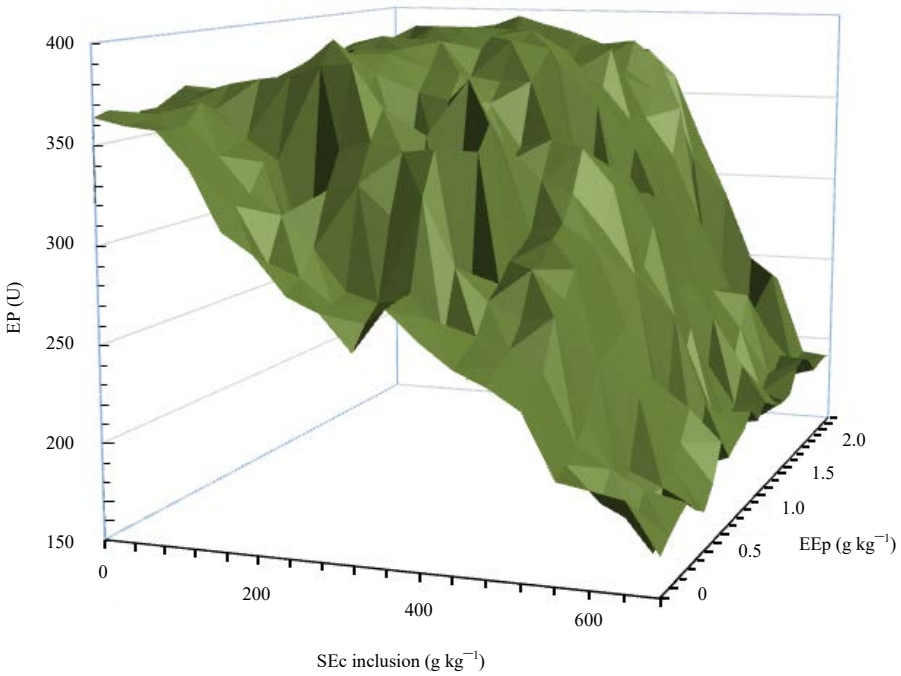

Fig. 1: Inclusion effect of *Secale cereale* (SEc) and pentosanases (EEp) in the feed of laying hens on egg production (EP), Interaction effect between the main factors on EP was observed ($p<0.000$), The experimental optimum level of inclusion for EP was 200 g kg^{-1} SEc and 1 g kg^{-1} EEp, with average production of 396 units (U), The contour area curvature analyses for EP (Spline Cubic Programme analysis) indicated an optimum response zone limited to the combinations 130-390 g kg^{-1} SEc and 0.38-1.57 g kg^{-1} EEp

(Table 3). The observation for the cell with 0 g kg^{-1} SEc with 2 g kg^{-1} EEp produced a relatively high value (47.21 kg), suggesting that increasing the EEp in the feed would apparently not have a negative effect on CC. The lowest mean value (29.15 kg) was observed corresponded to 600 g kg^{-1} SEc with 0 g kg^{-1} EEp. This demonstrates the negative effect on CC when the diet includes a feed with a high content of NSPs, as in this trial, the increase in the viscosity of the feed bolus affected voluntary feed consumption of fowls. All the cells with 600 g kg^{-1} SEc, independent of the proportion of EEp in the feed, presented the lowest values for CC in the experiment.

Figure 1 shows a graphic representation of the three dimensional response area observed for EP as a function of SEc and EEp. Analysing the spatial torsion of the surface plane and the singularity of the curvature, with inflexions for opposing trends, the presence of an interaction between the main factors and a trend zones can be observed. When the spatial relationship and the general trends for the variability of EP as a function of the proportion of EEp and SEc were described through the computer programme, a smoothing and torsion of the surface was observed. This produces a continuum of combinations from which the highest and lowest response zones for EP can be determined. Figure 1 shows optimum zones for combinations of EEp between 0.5 and 1.5 g kg^{-1}, with proportions of 200 and 400 g kg^{-1} SEc; the best response for EP is seen with the combination of 1 g kg^{-1} EEp with 200 g kg^{-1} SEc (396 U). This response surface analysis also shows a zone of low EP values at high proportions of SEc, including the lowest EP value in the trial (198.1 U) for 0 g kg^{-1} EEp with 600 g kg^{-1} SEc. In general, this response surface adopts a convex form with polarised expansion towards the low EP response zone and an optimum zone at the cusp, with a gentle slope downwards towards the higher levels of EEp and a slide towards minimum EP values in the region representing high proportions of SEc.

In the three dimensional spatial analysis of the main factors and CC (Fig. 2), irregular optimum zones were observed for medium-high EEp values and medium-low SEc. As with EP, a zone of low CC response to high levels of SEc was observed, independent of the levels of enzyme in the feed. Again, a convex general form was observed with an optimum level on the cusp, and surface torsions and curvatures with large zones of low response where the levels of SEc were high.

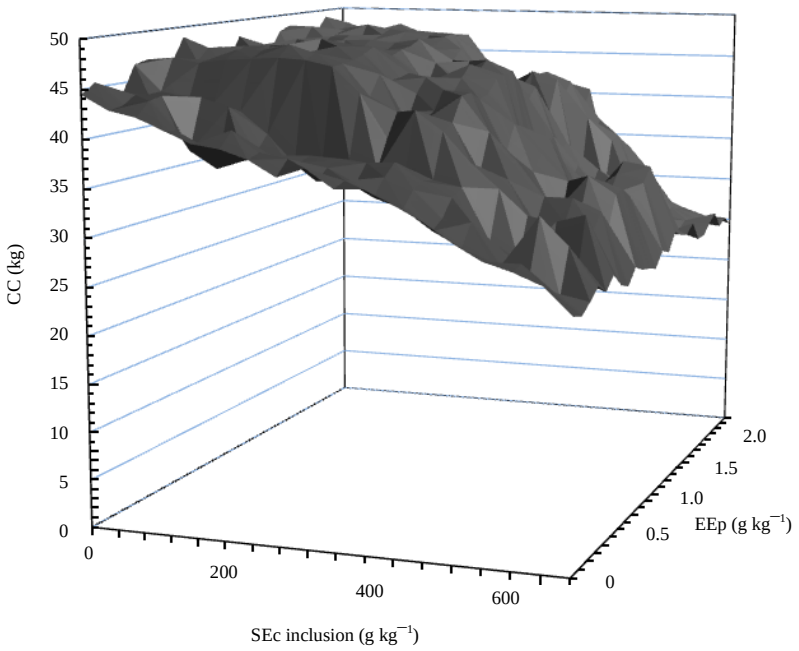

Fig. 2: Inclusion effect of *Secale cereale* (SEc) and pentosanases (EEp) in the feed of laying hens on concentrate consumption (CC), Interaction effect between the main factors on CC was observed ($p<0.000$), The empirical optimum for CC was 200 g kg^{-1} SEc with 0.5 g kg^{-1} EEp, with average consumption of 47.56 kg, The contour area curvature analyses for CC (Spline Cubic Programme analysis) indicated an optimum response zone to 120–350 g kg^{-1} SEc and 0.43-1.75 g kg^{-1} EEp

This spatial arrangement may indicate the presence of an interaction between the main factors affecting CC. An optimum response zone for the combinations of 0-200 g kg^{-1} SEc and 0.5-1.5 g kg^{-1} EEp can be observed.

The data plotted in Fig. 1 and 2enable to estimate a more precise optimum zone by generating superimposed ellipsoidal perimetric zones of increasing optimisation. Indeed, the contour area curvature analysis (Spline Cubic Programme) for EP indicated an optimum response zone limited to the combinations of 130-390 g kg^{-1} SEc and 0.38-1.57 g kg^{-1} EEp. For CC, this zone was found in the ranges of 120–350 g kg^{-1} SEc and 0.43-1.75 g kg^{-1} EEp.

DISCUSSION

The biological significance of the interaction observed in this investigation may be explained, in part, by the enzyme-substrate affinity and the kinetic conditions of the biochemical reactions in fowls' diets. It becomes more important if limiting enzyme- or substrate-related conditions become established (according to the reactive doses), producing a change in the trend of the EP response, i.e. for a certain level of EEp in the feed, the EP values would change with any change in the level of SEc. This enzymatic activity of EEp on SEc is based on the hydrolysis of the arabinoxylan bonds (depolymerisation of the NSPs complexes), allowing physiological optimisation of digestion and absorption of the biomolecules produced after degradation of the concentrate containing the different levels of SEc. Also, this molecular depolymerisation would facilitate degradation of the feed particles through synergetic action with other hydrolytic enzymes[16].

It has been shown that the degradation of NSPs would facilitate the endogenous enzymatic activity to digest the remaining nutrients in the diet[2,17,18]. When these chains are hydrolysed (*in vivo* or *in vitro*) by the action of enzymes exogenous to the fowl (cellulases, glucanases, pentosanases), the viscosity of the bolus diminishes significantly, reducing the possibility that it will form a gel, and thus avoiding the antinutritional action of these NSPs[3]. In this context, the pentosanes and β-glucan contents in SEc grains are among the highest of any cereal, with reported values of 89 and 12 g kg^{-1} DM, respectively. These values are higher than those observed in *Zea mays* L., *Sorghum vulgare* L., *Triticum aestivum* L. and *Hordeum vulgare* L., indicated as 43, 28, 61 and 76 g kg^{-1} DM (pentosanes), and 1, 1, 5 and 33 g kg^{-1} DM (β-glucans), respectively[1,19].

The increased levels of SEc in the diet negatively affected the EP response that was independent of the levels of EEp. The deleterious impact of the high levels of dietary SEc on the digestive and productive physiology of fowls can be observed (the lowest EP values were observed for 600 g kg^{-1} SEc). Although, in this study, CC did not affect the variations of EP, it may have affected the EP for nutritional reasons. In general terms, the combinations which produced a high CC also favoured EP. According to these findings, and considering the high contents of antinutritional factors in SEc (NSPs), a limit of 400 g kg^{-1} should be considered for the inclusion of this cereal in any diet for fowls, regardless of the proportion of EEp. There is probably an optimum limit to the enzyme activity of EEp on the substrate (SEc), rather than an ascending response curve as the limiting kinetic condition of the substrate increases.

When the results of the present research are compared with findings published in the literature, certain similarities may be observed. Various authors have reported the use of commercial enzymes to improve the digestibility of fowl feeds, including SEc, enzyme extracts prepared from the fungus *Aspergillus niger* figure largely among them. In the particular case of SEc, it has been reported that NSPs form a complex polymer in the cell wall, consisting of β (1→4) D-Xylan chains with α–(1→ 2, 3) L-arabinosyl ramifications, being the pentosanases highly effective in hydrolyzing these bonds[20]. In the majority of studies, favourable results were observed by adding this type of exogenous enzyme to the feed of productive fowl. These include diminished viscosity of the feed bolus and the faeces, increased conversion efficiency and a greater metabolizable energy contribution to the fowls, leading to a greater productive response[3,21]. In the context of the latter, in an investigation, improvements in conversion efficiency and concentrate consumption were reported when exogenous enzymes (β-Glucanases and xylanases) were added to SEc-based feeds for fattening of fowls, with proportions of 0 and 0.5 g kg^{-1} of concentrate. The positive effects on the speed of passage of the chyme through the fowl's digestive tract and a diminution of the bolus viscosity in small intestine were also reported ($p<0.001$)[22].

The contour area curvature analyses, for both EP and CC, allowed to establish quantitatively a smaller and more precise optimum response zone for combining SEc with EEp in the formulation of a feed. These results indicate the limits for the incorporation of SEc and EEp in the diet. In the case of EP, it could be suggested that the optimal content of SEc in a diet should not exceed 400 g kg^{-1} of inclusion, and should be accompanied by the addition of not less than 1.57 g kg^{-1} EEp. For CC, these values indicate a maximum inclusion of 350 g kg^{-1} SEc in a diet, always accompanied by a dose not less than 1.75 g kg^{-1} EEp. With these values, there would probably be no rejection of SEc by the hen during the consumption of the concentrate. The addition of EEp in the diets of the hens should always be considered when it is desired to control the antinutritional effects due to the incorporation of SEc in the concentrate. These results also allow to get a new base grid for treatment combinations for future research, in a more limited zone of the response curve area, both to optimize EP and CC.

CONCLUSION

The high level of *Secale cereale* inclusion in the diet affected negatively eggs production and concentrate intake. However, when pentosanases were included in the diet improve these variables. This answer can be explained by the enzymatic activity of pentosanase son *Secale cereale*, which is based on the depolymerisation of the NSPs complexes (antinutritional factors), allowing physiological optimisation of digestion and absorption of the biomolecules after concentrate degradation. The contour area curvature analyses, for both eggs production and concentrate intake, allowed to establish quantitatively a smaller and more precise optimum response zone for combining *Secale cereale* with pentosanases in the formulation of a feed.

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