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EFFECT OF TYPE OF WHEAT AND ADDITION OF ENZYMES ON SOME DIGESTIVE PARAMETERS AT DIFFERENT SAMPLING TIME. [1]

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ABSTRACT

Fifty one New Zealand x Californian growing rabbits were used to study the effect of sampling time, type of diet, type of wheat and enzyme addition on the weight, pH and amylase activity of digestive contents. Six diets were designed by substitution of a basal diet by 30% of two varieties of wheat (soft and hard wheat) and adding or not a commercial enzyme complex composed by amylase, xylanase, β-glucanase and pectinase. After 15 days eating the experimental diets, the animals were slaughtered at 10.00 or 20.00 h (morning and evening samples) and the digestive contents separated. Sampling time was the variable that most affected the digestive parameters studied. Stomach content was higher ($P<0.001$) in morning than evening samples, and the opposite effect was shown for intestinal and caecal content that resulted lower ($P<0.001$, $P=0.089$) during morning. The pH of stomach and intestine contents were lower and pH of caecal content was higher in the morning than evening samples. Likewise the amylase activity of intestinal content was much lower in the morning than in evening samples. The type of diet affected both diet intake and stomach content ($P=0.01$, $P<0.001$), pH of digestive contents ($P<0.05$), and amylase activity of caecal content ($P=0.067$). Substitution of basal diet by wheat produced an increase in intake and stomach content and amylase activity of caecum, whereas a decrease in the pH of the digestive contents. Type of wheat only affected amylase activity of stomach and caecum ($P=0.028$, $P=0.070$), being greater those of rabbits fed soft wheat based diets. Enzyme addition did not have any effect on the digestive parameters measured. Thus, the amylase activity of stomach was the same for rabbits fed on diets with or without exogenous amylase, and this activity was not recovered in small intestine or caecum.

INTRODUCTION

Wheat is the second cereal grain most commonly used, after barley, in rabbit diets in Spain. Diets with a high wheat content could cause greater amounts of starch in the caecum which serves as a substrate to bacterial growth, mainly in young animals because their enzymatic equipment is not completely developed (MAROUNEK et al., 1995). Therefore, enzyme addition could improve the digestion efficiency, increasing carbohydrates digestibility before the caecum which could avoid its effect on the diarrhoeas, as suggested by BORRIELLO and CARMAN (1983). Nevertheless, the stability of these exogenous enzymes to the low gastric pH is uncertain, according to works carried out with pigs and poultry (THACKER and BASS, 1996, ALMIRALL and ESTEVE-GARCIA, 1995). There is no information about the type of wheat and the effect of enzyme supplementation on several digestive traits in rabbits that could influence the nutritional value of diets with high dietary wheat content. On the other hand, BLAS, et al, (1988) observed an effect of time of sampling and of dietary starch on the amylase activity in saliva and pancreatic juice. Therefore, the aim of this work was to study the effect of type of diet, type of wheat and enzyme addition on the stomach, small intestine and caecal digesta content, pH and amylase activity in digestive tract of rabbits fed on diets with high wheat content, at different sampling time.

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MATERIAL AND METHODS

Feedstuffs and diets. Two varieties of wheat (*Yécora* and *Don Pedro*), each of *Triticum aestivum* (soft wheat) and *Triticum durum* (hard wheat), representative of the Spanish wheat production were used in this study. Their chemical composition are shown in Table 1.

Table 1. Chemical composition of wheat (%DM)

Type of wheat	*T. aestivum (Yécora)*	*T. durum (Don Pedro)*
Dry Matter	89.59	88.38
Ash	1.95	1.97
Crude Protein	16.81	17.94
Neutral Detergent Fibre	17.54	13.88
Acid Detergent Fibre	3.28	4.20
Acid Detergent Lignin	1.31	1.64
Starch	58.3	61.5

Six diets were formulated by substitution of a basal diet by 30% of both varieties of wheat. The ingredient composition of basal diet was: 25% barley, 35% alfalfa meal, 15% soyabean meal, 11% sunflower seed meal, 9.3% barley straw, 3.4% wheat bran, 1% calcium carbonate, 0.3% sodium chloride, 0.2% vitamin-mineral premix. These diets were supplemented or not with 0.2% of a commercial enzyme complex (Porzyme TX, Finnfeeds International Ltd, U.K.) composed of 4000 U/g xylanase, 150 U/g β-glucanase, 1000U/g α-amylase, and 25 U/g pectinase. Chemical composition of experimental diets is shown in Table 2.

Table 2. Chemical composition of experimental diets (% DM)

Diet	Basal	Soft wheat	Hard wheat
Dry Matter	91.40	90.84	90.75
Ash	8.83	7.13	7.71
Crude Protein	20.86	19.33	19.87
Crude Fibre	24.20	16.95	16.13
Neutral Detergent Fibre	37.30	31.30	29.82
Acid Detergent Fibre	21.87	16.52	16.32
Acid Detergent Lignin	4.82	3.70	3.67
Starch	12.26	26.07	27.04

Animals. Fifty one New Zealand x Californian growing rabbits of 39-50 days of age, were randomly allotted to the experimental diets (6-10 per diet). A cycle of 12 h of light and 12 h of dark was used throughout the experiment. Light was switched on at 7.30 h. Animals were given ad libitum access to diets. Intake was measured during the last four days of the experiment. After 15 days consuming the experimental diets, the animals were slaughtered (weight of rabbits 1609.5 ± 25.5 g) by cervical dislocation at two different times 10:00 and 20:00 h. The gastrointestinal tract was removed and weighed. The stomach, small intestine and caecum were weighed separately with and without their contents. Digestive contents were separated to measure their pH, and a sample was frozen and afterwards freeze-dried to measure enzyme activity.

Analyses. Analyses were conducted according to AOAC (1991) for DM, ash, CP, crude fibre (CF) and ether extract. Neutral detergent fibre, ADF and ADL were analysed sequentially (VAN SOEST et al., 1991). Starch was analysed using a kit of Boehringer Mannheim (nº 716251). Enzyme activity of the digestive contents were performed on freeze-dried material,

which was extracted with distilled water (50 mg lyophilised digesta in 1 ml of distilled water) for 1 h at 5ºC followed by centrifugation (3000 rpm) for 15 min. The supernatants were then collected for analysis of amylase activity. Amylase (EC 3.2.1.1) activity was determined using the Sigma amylase test kit (nº 577). The activity is expressed as units (which are defined mmol of P-nitrofenol released per minute at 30ºC) per gram of sample.

Statistical analyses were performed as a completely randomised design using the GLM procedures of SAS (1991). Orthogonal contrasts were performed to separate the effect of sampling time, diet, wheat type, enzyme addition and their interactions on the different digestive measures.

RESULTS

The weight of digestive organ contents, pH and amylase activity of digestive contents of growing rabbits fed on experimental diets are shown in Table 3. Dry matter intake during the four days previous to sampling was only affected by type of diet (P=0.011), rabbits fed basal diet showed higher intake (128.0 g/d) than those fed wheat based diets (115.2 g/d). Sampling time affected digestive content of stomach (P=0.0004), the stomach content being greater in the morning (0.44% DM/body weight) than in the evening (0.31%) samples. Weight of intestinal and caecal contents were also affected by the sampling time, but in the opposite way. Thus, rabbits slaughtered in the morning showed lower values than those slaughtered in the evening (0.20 vs 0.36%, P=0.0005 and 1.30 vs 1.46% P=0.089, for intestinal and caecal contents, respectively). Moreover, there was an effect of type of diet on the weight of stomach content (P=0.0009), showing higher values rabbits fed on basal diet (0.45%) than those fed on wheat based diets (0.33%). Type of wheat and enzyme addition did not significantly affect any digestive contents, although, an interaction of type of wheat by enzyme addition on the stomach content was found. Enzyme addition produced an increase in stomach content of animals fed on soft wheat based diet, and a decrease in those fed on hard wheat based diet .

The pH of the digestive contents (Table 3) were mainly affected by sampling time and type of diet. Thus, pH of stomach and small intestine contents were lower (P= 0.035, P = 0.004) in the samples collected in the morning than in the evening (1.07 vs 1.26; 7.27 vs 7.44 on average, respectively), whereas the opposite effect corresponded to the pH of caecal content (5.99 vs 5.62, P = 0.0001, for morning vs evening samples, respectively). Wheat inclusion decreased pH of stomach, small intestine and caecal contents with respect to the basal diets (1.29 vs 1.09, P = 0.02; 7.44 vs 7.29, P = 0.01 and 5.91 vs 5.73, P = 0.005 on average, respectively). The enzyme addition also caused a reduction (0.11 points, P=0.06) in the pH of stomach.

The amylase activity (Table 3) of intestinal content was much lower (P=0.002) in the morning than in the evening samples (4.54 vs 8.75 U/g on average, respectively). The amylase activity of caecal and stomach content was affected by type of diet or type of wheat. Thus, the dietary starch level was positively related to the amylase activity of caecal content. Rabbits fed on wheat based diets showed higher values than those fed on basal diet (1.42 vs 0.83 U/g, on average, respectively; P = 0.07). The source of starch and not the level affected (P = 0.03) the amylase activity in stomach content. Rabbits fed on soft wheat based diets showed lower amylase activity in stomach than those fed on hard wheat based diets (0.025 vs 0.046 U/g, on average, respectively). The same trend (P = 0.07) was found in the amylase activity of caecal content (1.07 and 2.01 U/g, on average, for rabbits fed soft and hard wheat based diets, respectively). The two triple interactions, sampling time by wheat type by enzyme addition (P=0.004) and sampling time by type of diet by enzyme addition (P=0.064), were observed on the caecal contents.

DISCUSSION

Sampling time was the variable that showed the highest influence on the digestive parameters studied. The effect of sampling time on the weight of digestive contents is related to the marked circadian rhythms of caecotrophy and feed intake observed in rabbits (CARABAÑO and PIQUER, 1998) and to the rate of passage of digesta throughout the different organs. Thus, soft faeces are excreted during morning (84% from 8.00 to 16.00 h) whereas feed intake occurs mainly during the evening and night (86% from 16.00 to 8.00 h) (CARABAÑO and MERINO, 1996). Taking into account the passage of feed through stomach (from 3 to 6 h, CARABAÑO and PIQUER, 1998), both soft faeces and feed are present in the stomach content during the morning, contributing to its higher weight (41% in the current study), whereas in late evening (20.00h) only feed is present. FRAGA et al (1984) also observed greater stomach contents (30%) during the morning. The opposite tendency was observed in small intestine content (evening samples were 80% higher than morning samples). However, the interpretation of this results is not clear, because of the transit of digesta through the small intestine is very fast (40 to 100 minutes, CARABAÑO and PIQUER, 1998) and then liable to important changes in short periods of time. Caecal content is also affected by sampling time, because during the morning caecum is being emptied in the form of soft faeces excretion. According to CARABAÑO and PIQUER, (1998) diurnal variation up to 30% of the weight of caecal content can be observed. The evolution of pH of stomach content during the day was studied by FRAGA et al (1984), showing a high variability, as a consequence of the different proportion of soft faeces in the stomach content and of its degree of destruction. Thus, the maximum pH had been observed at 12:00h, with the greatest proportion of soft faeces. In the current study, lower values of pH were observed during morning sampling, but, samples were collected (at 10.00h) before the expected highest soft faeces proportion. The pH of small intestine contents was positively correlated ($r=0.30$ $P=0.03$) with gastric pH, following the same trend. Caecal pH resulted lower in the evening samples due to the greater concentration of volatile fatty acids as a consequence of an increase both in substrate and microbial population (CARABAÑO and PIQUER, 1998). The greatest amylase activity in the small intestine during the evening (intake period) is related to the stimulation of pancreas gland by digesta in duodenum activating the secretion of juice (BLAS et al. 1988). In fact, these authors found twice more specific amylase activity in the pancreas juice of rabbits 150 min after meal than those fasted.

The type of diet affected pH of digestive contents and amylase activity in the caecum. Gastric pH was lower in rabbits fed high starch diets than those fed the basal diet, unlike DE BLAS et al (1986) who did not find differences in pH of stomach among diets ranging in starch content from 13 to 30%. Similarly, rabbits fed wheat based diets showed lower caecal pH, which could be related to a higher amount of fermentable carbohydrates in the caecum; in fact, a tendency to a higher NDF digestibility was observed for these diets (SEQUEIRA and VILLAMIDE, 1999). Amylase activity in the caecum was greater in rabbits fed wheat based diets and mainly those fed hard wheat. BLAS (1986) found a direct relation of dietary starch level on amylase activity of caecum. The highest values observed in hard wheat could be related to a higher amount of starch in caecum and its lower pH, although no differences were found for starch digestibility, because the faecal starch digestibility was almost total (99.4%, SEQUEIRA and VILLAMIDE, 1999). Positive correlations among pH of stomach and intestinal contents and their amylase activity were observed ($r=0.26$, $P=0.06$; $r=0.28$, $P=0.05$, respectively). Amylase activity of stomach content was very low (about 200 times lower than intestinal content), and was only significantly affected by wheat type. The amylase of stomach comes from saliva, microbial population of soft faeces, or exogenous addition to diet.

The two latter were more stable at acidic pH (BLAS, 1986), so the ingestion of caecal content with higher amylase activity throughout caecotrophy could explain this effect.

Enzyme addition did not affect significantly any of parameters measured except a decrease in pH of stomach contents, which could be due to a relatively higher amount of carbohydrates available to be fermented. The addition of exogenous enzyme did not increased amylase activity in stomach, probably as a consequence of the low pH observed there (1.17). Thus, assays carried out to determine the stability of added enzymes to the different pH of digestive tract (THACKER and BASS, 1996) showed that pH below 3.5 were clearly detrimental to β-glucanase and xylanase activity, and they only recovered partial activity (26 and 84% for β-glucanase and xylanase, respectively) in the duodenum of pigs (at most adequate pH) because of the protective effect of feed on added enzyme. Nevertheless, according to the results obtained in the current study, the exogenous amylase activity was not recovered either in the small intestine or caecum content, because no differences among amylase activity were shown between animals supplemented or not with enzymes.

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Table 3. Effect of sampling time, diet, type of wheat and enzyme addition on DM intake, weight of digestive organ contents, amylase activity and pH of digestive contents of growing rabbits.

Diet	Sampling	Basal		Soft Wheat		Hard Wheat			Significance of Orthogonal Contrasts			
Enzyme	Time	-	+	-	+	-	+	SEM	Time[1]	Diet[2]	Wheat type[3]	Enzyme[4]
n		10	10	8	8	9	6					
Dry Matter Intake (g/d)	M	122.7	131.9	118.1	116.7	111.1	115.6	5.37	-	0.011	0.479	0.356
Weight of digestive contents (% DM/Body Weight)												
Stomach content[5]	M	0.582	0.460	0.343	0.482	0.375	0.379	0.04	0.0004	0.0009	0.564	0.695
	E	0.417	0.357	0.213	0.308	0.313	0.174					
Small Intestine content	M	0.173	0.225	0.178	0.18	0.193	0.292	0.05	0.0005	0.147	0.882	0.728
	E	0.456	0.425	0.336	0.335	0.308	0.266					
Caecal Content	M	1.169	1.235	1.296	1.363	1.309	1.433	0.11	0.089	0.479	0.275	0.799
	E	1.648	1.291	1.514	1.689	1.345	1.266					
pH of digestive contents												
Stomach	M	1.18	1.00	1.25	0.83	1.13	1.00	0.09	0.035	0.019	0.919	0.055
	E	1.43	1.47	1.14	1.10	1.19	1.03					
Small Intestine	M	7.35	7.28	7.23	7.40	7.23	7.06	0.06	0.004	0.011	0.193	0.513
	E	7.57	7.54	7.40	7.31	7.35	7.34					
Caecum	M	6.17	6.05	6.02	5.90	5.94	5.80	0.09	0.0001	0.005	0.704	0.263
	E	5.80	5.70	5.38	5.64	5.66	5.39					
Amylase Activity in digestive contents (U/g)												
Stomach	M	0.053	0.019	0.035	0.021	0.053	0.061	0.01	0.529	0.747	0.028	0.173
	E	0.046	0.024	0.019	0.025	0.056	0.037					
Small Intestine	M	3.53	5.74	4.24	3.81	5.81	3.90	1.56	0.002	0.657	0.119	0.640
	E	9.83	6.04	8.20	6.10	10.34	12.69					
Caecum [6]	M	0.38	0.36	0.56	1.06	2.97	0.85	0.44	0.180	0.067	0.070	0.284
	E	2.13	0.36	2.02	0.64	1.00	3.23					

[1] Time: Morning (M) vs Evening (E) sampling; [2] Diet: Basal vs Wheat based diets, [3] Wheat type: Soft vs Hard wheat based diets, [4] Enzyme: without (-) vs with (+) enzyme,
[5] Interaction Enzyme x wheat type: P=0.035, [6] Interaction Time x wheat type x enzyme: P=0.004, Time x enzyme x diet: P=0.064