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Influence of non-starch polysaccharide-degrading enzymes on growth performance, blood parameters, and carcass quality of broilers fed corn or wheat/barley-based diets¤

Influencia de enzimas degradadoras de polisacáridos no amiláceos en el crecimiento, parámetros sanguíneos y calidad de la canal de pollos de engorde alimentados con dietas a base de maíz o trigo/cebada

Influência de enzimas exógenas degradaroras de polissacarídeos não amiláceos no crescimento, parâmetros sanguíneos e qualidade de carcaça de frangos de corte alimentados com dietas à base de milho e de trigo/cevada

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Abstract

Background: Although the use of non-starch polysaccharide-degrading enzymes (NSPases) in corn, oat, rye, barley or wheat-based broiler diets has already been researched for some years, little attention has been given to the mixture of wheat and barley, as basic raw materials for broiler feed. **Objective:** To evaluate the effect of different inclusion levels of commercial NSP enzymes in corn or in the mixture of wheat/barley-based diets on growth performance, carcass quality and blood parameters of broilers. **Methods:** Three hundred 1 d-old male broiler chicks (Ross-308) were fed two basal diets (corn and a wheat/barley-based diets), two commercial feed enzymes (Kemin® and Rovabio®), and two enzyme levels (0.025 and 0.05%) in a 2×2×2 factorial arrangement, from 1 to 42 d of age. **Results:** Overall, birds fed corn-based diets with or without enzyme supplementation consumed more feed ($p<0.05$) over the entire experiment, experienced higher weight gain ($p<0.05$) and lower feed conversion ratio (FCR; $p<0.05$) when compared with wheat/barley-based diet. Notwithstanding, FCR

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did not improve in birds fed corn-based diets with enzymes, while gain and FCR improved (p<0.05) feeding wheat/barley-based diets with 0.05% NSPases. Economical traits of carcass were not affected (p>0.05) by the treatments, while blood biochemistry parameters, such as glucose, VLDL and HDL changed (p<0.05) when enzymes were supplied. **Conclusion:** Our results show bio-efficacy of feeding xylanases and glucanases in wheat/barley based-poultry diets, rich in NSPases, which could translate into economic benefits.

Keywords: *carcass characteristics, cereals, exogenous NSP-ases, feed efficiency, poultry nutrition.*

Resumen

Antecedentes: Aunque el uso de enzimas degradadoras de polisacáridos no amiláceos (NSPasas) en dietas a base de maíz, avena, centeno, cebada o trigo ha sido investigado, se ha prestado poca atención a la mezcla de trigo y cebada como materias primas básicas para la alimentación del pollo de engorde. **Objetivo:** Evaluar el efecto de la inclusión de diferentes niveles de enzimas NSP comerciales en dietas basadas en maíz y en la mezcla de trigo/cebada sobre el crecimiento, calidad de la canal y metabolitos sanguíneos del pollo de engorde. **Métodos:** Trescientos pollos machos de 1 d de edad (Ross-308) fueron alimentados con dos dietas (una dieta a base de maíz y una dieta basada en trigo/cebada), dos enzimas comerciales (Kemin® y Rovabio®), y dos niveles de enzimas (0,025 y 0,05%) en un arreglo factorial de 2×2×2, desde el d 1 al 42 de edad. **Resultados:** En general, las aves alimentadas con dietas a base de maíz (con o sin suplementación enzimática) consumieron más alimento (p<0,05) durante todo el experimento, mostraron mayor aumento de peso (p<0,05) y menor FCR (p<0,05) en comparación con la dieta basada en trigo/cebada (con o sin suplementación enzimática). Sin embargo, cuando se proporcionaron las enzimas, la FCR no mejoró en las aves alimentadas con la dieta a base de maíz. Por el contrario, en las aves alimentadas con trigo/cebada aumentó el peso corporal y la conversión alimenticia mejoró (p<0,05) con la inclusión de 0,05% NSPasas. Los tratamientos dietarios no afectaron (p>0.05) las características económicas de la canal, mientras que parámetros de bioquímica sanguínea como glucosa, colesterol, VLDL y HDL cambiaron (p<0,05) al incorporar enzimas en la dieta. **Conclusión:** Los resultados muestran la bioeficacia de xilanasas y glucanasas en dietas avícolas a base de trigo/cebada, ricas en NSPasas, lo que se podría traducir en beneficios económicos para el productor.

Palabras clave: *características de la canal, cereales, eficiencia alimenticia, NSP-asas exógenas, nutrición avícola.*

Resumo

Antecedentes: Embora o uso de enzimas degradadoras de polisacarideos não amiláceos (NSPasas) em dietas de frangos de corte à base de milho, aveia, centeio, cevada ou trigo já venha a ser estudada há vários anos, pouca atenção tem sido dada à mistura de trigo e cevada como matérias-primas básicas para a ração de frangos. **Objetivos:** Avaliar o efeito de diferentes níveis de enzimas NSP comerciais em dietas à base de milho e à base da mistura de trigo/cevada sobre o desempenho produtivo, a qualidade da carcaça e os parâmetros bioquímicos sanguíneos em frangos de corte. **Métodos:** Trezentos frangos de corte machos de 1 d de idade (Ross-308) foram alimentadas com duas dietas basais (uma à base de milho e outra à base de trigo e cevada), dois produtos enzimáticos comerciais (Kemin® e Rovabio®) e dois níveis dessas enzimas (0,025 e 0,05%), num arranjo fatorial 2×2×2, de 1 a 42 d de idade. **Resultados:** Em geral, as aves alimentadas com dietas à base de milho (com ou sem suplementação enzimática) consumiram mais alimentos (p <0,05) ao longo do experimento, apresentaram maior ganho de peso (p <0,05) e menor RRF (p<0,05) em comparação com a dieta à base de trigo/cevada (com ou sem suplementação enzimática). No entanto, quando as enzimas foram fornecidas, o FCR não melhorou em aves alimentadas com a dieta à base de milho. Em contraste, em aves alimentadas com trigo/cevada, o peso corporal aumentou e a conversão alimentar melhorou (p<0,05) com inclusão de 0,05% de NSPasas. Os tratamentos dietéticos não afetaram as características econômicas da carcaça (p>0,05), enquanto os parâmetros bioquímicos do sangue, como glicose, colesterol, VLDL e HDL, mudaram (p<0,05) ao incorporar enzimas na dieta. **Conclusão:** Os resultados confirmam a bioeficiência da inclusão de xilanases e glucanases nas dietas à base de trigo e cevada, ricas em NSPasas, o que poderá trazer benefícios económicos para o produtor.

Palavras chave: *características de carcaça, cereais, eficiência alimentar, NSP-asas exógenas, nutrição avícola.*

Introduction

In order to use the high genetic potential of modern broiler strains, nutrient requirements, and nutritional management of poultry have changed (Ravindran, 2013a). Feed represents the most significant component of poultry production costs. Poultry diets are primarily based on corn and soy. The continuous rise of corn and soy prices worldwide generated a growing use of alternative cereals in broiler diets, such as wheat and barley. However, the cell wall of these cereals are rich in insoluble and soluble polysaccharides other than starch. These complex non-starch polysaccharides (NSP) are a heterogeneous group of compounds that include cellulose, pectins, β-glucans, pentosans, heteroxylans, and xyloglucan, which cannot be hydrolyzed by the endogenous digestive enzymes of humans and monogastric animals (Kumar *et al.,* 2012).

Soluble NSP increase digest viscosity due to their high capacity to absorb water and gelatinize the intestinal tract contents of birds (Choct *et al.*, 2010). This phenomenon disturbs digestion with consequences for feed energy, feed efficiency (Choct, 2006), and broiler performance (Chandra Shekhar *et al*., 2014). Hence, cereal grains high in non-starch polysaccharides (NSP) such as wheat (cellulose, and therefore largely insoluble) and barley (largely soluble) exhibit poor nutritional value (low apparent metabolizable energy, AME). Along with this, high dietary levels of NSP lead to high nitrogen and phosphorus excretion, which is a major environmental concern in densely populated regions.

Wheat and corn are rich in arabinoxylan, whereas barley has high levels of β-glucan (Knudsen, 2014). The anti-nutritional properties –expressed by water-soluble NSPs, such as pentosans (arabinoxylans) and β-glucans– can be reduced by including NSP degrading enzymes (xylanase-glucanase and cellulase) in broiler diets. The NSP degrading enzymes (NSPases) are known to increase the digestibility of raw materials for monogastrics and a combination of NSPases, amylases and proteases help digesting feed ingredients, thereby increasing the use of nutrients and the energy available for growth (Oluyinka *et al.,* 2008) and production (Friesen *et al.,* 1992; Zanella *et al.,* 1999; Lázaro *et al.,* 2003).

Although the effect of enzyme supplementation in wheat and barley-based diets for broilers has been studied for the last decades, controversial results have been published (Mathlouthi *et al*., 2002; 2003a; 2003b). Therefore, the objective of this study was to evaluate the effect of different levels (0, 0.025, and 0.05%) of two commercial enzyme mixtures (Kemin®, glucanase, amylase, xylanase, and bacillolysin; Kemin Industries, Inc., EMEA, Herentals, Belgium, and Rovabio®, glucanase and xylanase; Adisseo France S.A.S., Antony, France) on performance, blood constituents, and carcass characteristics of broiler chicks fed corn and wheat/barley diets during a 6-wk rearing period. Birds were fed the experimental diets from hatch to 42 d of age.

Materials and methods

Ethical considerations

This experiment was conducted during July and August of 2013 in the poultry facility of the Islamic Azad University, in Rasht, Iran (37°14’37.15’’N and 49°34’39.42’’E). All procedures were approved by the Scientific Board of the Islamic Azad University and the study was conducted according to the International Guidelines for research involving animals (Directive 2010/63/EU).

Housing and management

A total of 300 male Ross 308 (Aviagen, Newbridge, Scotland, UK 35805) chicks were randomly allocated to 10 dietary treatments with 3 replicate pens/diet and 10 broiler chicks/pen. Birds were housed in an environmentally controlled system and growing conditions were similar in all treatments. Birds were vaccinated against infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and Gumboro virus (GV) on varying time intervals. The infectious bronchitis virus strain H120 was provided at d 1 and d 19. The Newcastle disease virus strain *Viscerotropic velogenic* was provided at d 1, 11, and 19. The Gumboro virus was provided at d 14 and 25. Vaccines were prepared per vendor recommendation and were supplied via drinking water after a period of water removal for 3 h.

Dietary treatment

The ingredients and nutritional composition of the diets used are presented in Table 1.

The two basal diets used were corn-based and wheat/barley-based. These diets were formulated to be isoenergetic and isonitrogenous and met or exceeded Ross 308 catalogue recommendations (Aviagen, 2007). Starter diets were offered from d 1 to 14, grower diets from d 15 to 28 and the finisher diets from d 29 to 42. The experimental design was completely randomized in a 2×2×2 factorial arrangement with the two basal diets (corn-based or wheat/barley-based), two commercial enzymes (Kemin® or Rovabio®) and two enzyme levels (0.025 or 0.05%). Thus, broilers were assigned to 10 dietary treatments (Table 2): Two control diets [i.e. without any enzyme supplementation (T1, diet based on corn without enzyme and T6, diet based on wheat-barely without enzyme)], a corn-based diet in which 0.025% Kemin® was added (T2), a corn-based diet in which 0.05% Kemin® was added (T3), a corn-based diet in which 0.025% Rovabio® was added (T4), a corn-based diet in which 0.05% Rovabio® was added (T5), a wheat/barley-based diet in which 0.025% Kemin® was added (T7), a wheat/barley-based diet in which 0.05% Kemin® was added (T8), a wheat/barley-based diet in which 0.025% Rovabio® was added (T9), a wheat/barley-based diet in which 0.05% Rovabio® was added (T10).

Table 1. Feed ingredients and nutrient analysis of diets used during the experiment.

	Starter period (1st-14th d of age)		**Grower period (15th-28th d of age)**		**Finisher period (29th-42nd d of age)**	
Ingredient (%)	*Corn*	*Wheat/barley*	*Corn*	*Wheat/barley*	*Corn*	*Wheat/barley*
Corn	60.0	0	60.0	0	60.0	0
Wheat	0	30.0	0	30.0	0	30.0
Barley	0	30.0	0	30.0	0	30.0
Wheat bran	0	0	0	0	5.57	7.53
Soybean meal	21.27	20.01	22.79	21.44	17.71	10.46
Corn gluten meal	12.44	10.26	8.92	6.74	8.00	9.55
Soybean oil	0.89	4.42	3.53	7.07	4.06	7.80
L- Lysine-Hydro-Chloride	0.06	0.06	0.04	0.03	0.02	0.04
DL-Methionine	0.17	0.21	0.12	0.15	0.04	0.02
Ca:22% P:18% ($CaHPO_4$)	2.13	2.04	1.87	1.78	2.02	1.76
CaCO3	2.10	2.19	1.89	1.98	1.75	2.04
NaCl	0.34	0.31	0.34	0.31	0.33	0.30
Vitamin mixture[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mixture[2]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Nutrient analysis						
Energy (ME; KJ/Kg)	11 954.80	11 954.80	12 540.00	12 540.00	12 665.40	12 665.40
Crude protein (%)	22.41	22.41	20.95	20.95	19.00	19.00
Lysine (SID, %)	1.27	1.27	1.14	1.14	0.95	0.95
Met + Cys (SID, %)	1.03	1.03	0.89	0.89	0.76	0.76
Crude fiber (%)	2.05	3.44	2.05	3.45	2.81	3.87
Calcium (%)	0.95	0.95	0.86	0.86	0.85	0.85
Available phosphorus (%)	0.47	0.47	0.43	0.43	0.43	0.43
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15

[1]Vitamin A: 5000 IU/g; Vitamin D3: 500 IU/g; Vitamin E: 3 mg/g; Vitamin K3: 1.5 mg/g; Vitamin B2: 1 mg/g. [2]Calcium pantothenate: 4 mg/g; Niacin: 15 mg/g; Vitamin B6: 13 mg/g; Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g.

Table 2. Summary of studied treatments.

	Corn						Wheat/barley					
	Kemin®			Rovabio® (%)			Kemin® (%)			Rovabio® (%)		
Type of enzyme (%)	0	0.025	0.05	0	0.025	0.05	0	0.025	0.05	0	0.025	0.05
Treatment	T1	T2	T3	T1	T4	T5	T6	T7	T8	T6	T9	T10

Enzyme mixtures

The exogenous enzymes mixtures used were the two commercial multi-enzyme preparations, Kemin® (Kemin Industries, Inc., EMEA, Herentals, Belgium) and Rovabio® (Adisseo France S.A.S., Antony, France). The Kemin® mixture includes five active substances: Endo-1,3(4)-ß-glucanase, endo-1,4-ß-glucanase (cellulase), α-amylase, endo-1,4- ß-xylanase and bacillolysin (protease) with a guaranteed minimum enzyme activity of 2,350, 180,00, 400, 35,000, and 1,700 U/g, respectively. The Rovabio® mixture includes endo-1,3(4)-ß-glucanase (30,000 U/g) and endo-1,4-ß-xylanase (22,000 U/g) produced by a non-genetically modified strain of *Penicillium funiculosum* Pf 8/403 (International Mycological Institute, under the number IMI 378536).

Performance traits

Feed intake (FI, g/bird/period) and body weight gain (BWG, g/bird/period) were recorded for the period at the beginning of the experiment (d 1) until the end of the starter period (14th d of age), the grower period (15th-28th d of age) and finisher period (29th-42nd d of age). Feed efficiency (referred to as feed conversion ratio —FCR) was calculated by dividing feed intake by body weight gain. On the final day of the experiment, at 42 d-of-age, one bird from each replicate (three from each treatment) was randomly selected and slaughtered, plucked and eviscerated. Economically valuable carcass and gastrointestinal segments were removed and weighted. Organ weights were used to calculate percent of total body mass associated with each organ. Liver, pancreas, gizzard, and abdominal fat were removed to obtain the eviscerated carcass. The *pectoralis major* and *peroneous longus muscles* were excised from breast and drumstick for analysis.

Sampling and blood parameters

At the end of the experiment, at 42 d-of-age, 1 bird/replicate, totaling 3 birds/treatment, was randomly selected for blood collection. Blood samples (1.5 mL/bird) were collected from the wing vein using a 2 mL syringe and left to stand at 30 °C to allow clotting and clot retraction. The serum that remained after clotting was centrifuged at 3000 rpm for 10 min at room temperature. Samples were chilled until analyzes.

Blood parameters analyzed in serum were glucose (GLU), total triglycerides (TG), total cholesterol (TC), very low density lipoprotein (VLDL), high density lipoprotein (HDL), total protein (TP), albumin (Alb), uric acid (UAc) and alkaline phosphatase (ALP). The concentrations for these parameters were determined by routine methods using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran), according to the manufacturer's instructions.

The GLU was measured by a glucose-oxidase photometric assay, based on the combined action of glucose oxidase (GOD) and peroxidase (POD; COD-POD assay; Barham and Trinder, 1972). In this assay glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide reacts with phenol and 4-aminoantipyrine in the presence of peroxidase to form a quinoneimine dye (Trinder's reaction). Intensity of the pink color formed is proportional to glucose concentration.

The levels of serum TC, HDL, VLDL, and TG were determined using enzymatic methods (Teif AzmoonPars, Co., Tehran, Iran). The colorimetric determination of cholesterol in blood serum samples by the CHOD-PAP assay involved the hydrolysis of cholesterol esters to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-in-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500 nm (Allain *et al.,* 1974). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) was measured directly after VLDL and/or LDL precipitation by the action of a precipitating reagent (heparin, for VLDL determination, and polyethylene glycol (PEG), for HDL determination) on the serum. When heparin was used as the precipitation reagent all the supernatant contained VLDL and HDL, which were measured by cholesterol CHOD/ PAP method. Low-density lipoprotein was equal to the difference between total cholesterol (TC) and cholesterol in the supernatant. When PEG was used as the precipitation reagent, all the VLDL and the LDL were precipitated. The HDL remained in the supernatant and was then assayed as a sample for cholesterol (CHOD/ PAP method).

Plasma triglycerides (TG) were measured using a series of coupled reactions in which triglycerides are hydrolyzed by lipase, and the released glycerol is assayed in a reaction catalyzed by glycerol kinase and L-alpha-glycerol-phosphate oxidase in a system that generates hydrogen peroxide. The hydrogen peroxide is monitored in the presence of horseradish peroxidase with 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone as the chromogenic system (Fossati and Prencipe, 1982).

Blood serum albumin (Alb) was determined based on the bromocresol green method (Doumas *et al.,* 1971), whilst UAc was determined by enzymatic methods using the uricase-TOOS method (Kayamori *et al*., 1997), TP was assayed by the Biuret method (Gornall *et al*., 1949). The serum activity of ALP was determined enzymatically using commercial kits (Teif Azmoon Pars, Co., Tehran, Iran) according to the method described by Bessey *et al.* (1946).

Statistical analysis

Data were analyzed by a General Linear Model using a 2×2×2 factorial arrangement (Statistical Package for the Social Sciences, SPSS, 1997; Base 7.5 for Windows. Chicago, IL). The statistical model used was as follows:

$$Y_{ijk\,l} = \mu + a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkl},$$

In which the value of each observation (Y) relies on the population effects average (μ) plus the diet effect (α_i, basal diet; corn or wheat and barley-based), the type of enzyme effect (β_j, commercial feed enzyme type; Kemin® or Rovabio®), the level of enzyme effect (c_k, commercial feed enzyme level; 0.025 or 0.05%), their interactions [$(\alpha\beta)_{ij}$, $(\alpha\chi)_{ik}$, $(\beta\chi)_{jk}$, $(\alpha\beta\chi)_{ijk}$], and the experimental error (ε). The Duncan post hoc test was used if the initial test result was significant at $p \leq 0.05$.

Results

Feed efficiency

Results of feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) are summarized in Tables 3, 4, and 5, respectively.

Concerning birds fed corn-based diet supplemented with 0.025 and 0.05% Kemin® or 0.025 and 0.05% Rovabio® enzymes, the results showed that during starting and growing periods FI was significantly lower ($p<0.05$) than in birds of the corn-fed control group. This was in contrast with the significantly ($p<0.05$) increased FI of birds fed wheat/barley-based diet supplemented with 0.05 Kemin® or 0.05% Rovabio® enzyme mixtures (Table 3).

The increase in FI observed in birds fed wheat/barley-based diet supplemented with 0.05 of Kemin® or 0.05% of Rovabio® enzyme mixtures was accompanied by an increase in BWG ($p<0.05$), while with corn diets the lower FI also resulted in lower BWG, which was particularly evident during the starter period (Table 4).

During the starter and grower periods, the FCRs for the wheat/barley-based diets supplemented with 0.05% enzyme mixture were significantly lower ($p<0.05$) than for the wheat/barley control diet. For birds fed corn-based diets supplemented with NSPases, the FCRs were significantly higher ($p<0.05$) than in the corn-based control diet during the first 14 d period (Table 5). Nevertheless, when compared with the corn-based control diet, the differences in the FI and the BWG observed in birds fed corn-based diets supplemented with commercial enzymes disappeared with age, as did the differences in the FCR. These results suggested a small bio-efficacy of exogenous xylanases and glucanases in corn diets during the earlier stages of bird's life.

Table 3. Effects of supplementing corn and a wheat/barley-based diet with enzyme mixtures (Kemin® and Rovabio®, at two dose levels each) on feed intake (FI; g/chick/period; mean ± sem).

Dietary treatment	Trait	Starter period (1st-14th d of age)	Grower period (15th-28th d of age)	Finisher period (29th-42nd d of age)	Total period (1st-42nd d of age)
Basal diet	Wheat-barely	358.483^{a} ± 2.683	1341.475^{b} ± 8.347	2906.875^{b} ± 56.491	4606.833^{b} ± 61.489
	Corn	362.016^{a} ± 2.683	1400.975^{a} ± 8.347	3374.516^{a} ± 56.491	5138.016^{a} ± 61.489
Enzyme type in diet	Kemin®	361.166^{a} ± 2.683	1363.725^{a} ± 8.347	3174.033^{a} ± 56.491	4898.891^{a} ± 61.489
	Rovabio®	359.333^{a} ± 2.683	1378.725^{a} ± 8.347	3107.358^{a} ± 56.491	4845.958^{a} ± 61.489
Enzyme level (% in diet)	0.025	355.708^{a} ± 2.683	1353.900^{b} ± 8.347	3176.191^{a} ± 56.491	4885.766^{a} ± 61.489
	0.05	364.791^{a} ± 2.683	1388.550^{a} ± 8.347	3105.200^{a} ± 56.491	4859.083^{a} ± 61.489
T1: Corn without enzyme		404.667^{a} ± 3.762	1428.300^{a} ± 8.475	3485.266^{a} ± 8.111	5318.233^{a} ± 8.775
T2: Corn with Kemin® (0.025%)		368.100^{b} ± 3.762	1398.100^{b} ± 8.475	3414.266^{a} ± 8.111	5180.333^{ab} ± 8.775
T3: Corn with Kemin® (0.05%)		361.166^{bcd} ± 3.762	1402.366^{b} ± 8.475	3349.366^{a} ± 8.111	5112.900^{ab} ± 8.775
T4: Corn with Rovabio® (0.025%)		365.300^{bc} ± 3.762	1395.100^{b} ± 8.475	3421.166^{a} ± 8.111	5181.566^{ab} ± 8.775
T5: Corn with Rovabio® (0.05%)		353.500^{cde} ± 3.762	1408.333^{d} ± 8.475	3313.266^{a} ± 8.111	5077.266^{b} ± 8.775
T6: Wheat-barely without enzyme		335.000^{f} ± 3.762	1304.466^{d} ± 8.475	2925.166^{bc} ± 8.111	4564.633^{c} ± 8.775
T7: Wheat-barely with Kemin® (0.025%)		342.166^{ef} ± 3.762	1310.133^{d} ± 8.475	2931.233^{bc} ± 8.111	4583.533^{c} ± 8.775
T8: Wheat-barely with Kemin® (0.05%)		373.233^{b} ± 3.762	1344.300^{c} ± 8.475	3001.266^{b} ± 8.111	4718.800^{c} ± 8.775
T9: Wheat-barely with Rovabio® (0.025%)		347.266^{def} ± 376.2	1312.266^{d} ± 8.475	2938.100^{bc} ± 8.111	4597.633^{c} ± 8.775
T10: Wheat-barely with Rovabio® (0.05%)		371.266^{b} ± 3.762	1399.200^{b} ± 8.475	2756.900^{c} ± 8.111	4527.366^{c} ± 8.775

Growth period values (within columns) with different superscripts letters ($^{a, b, c, d, e, f}$) differ significantly (p<0.05).

The birds fed corn-based diets showed the highest BWG throughout the experiment (Table 4). In wheat/barley-based diets, supplementation levels of 0.05% Kemin® (T8) and both 0.025 and 0.05% Rovabio® significantly (p<0.05) improved the BWG when compared with the wheat/barley-control group (p<0.05). Furthermore, both 0.025 and 0.05% Kemin® supplemented chicks showed a BWG that was not different (p>0.05) from birds supplemented with 0.025 or 0.05% Rovabio®.

At d 42, results showed that birds fed corn-based diets with Kemin® and Rovabio® had no improvement in feed efficiency (Table 5). Indeed, FCR appeared to be increased, but the two enzyme mixtures did not cause significant changes (p>0.05). Overall, birds fed the wheat/barley-based diets showed greater FCR than the birds fed corn-based diets. Significant differences were observed for birds fed wheat/barley-based diets with 0.025% of Kemin® or Rovabio® when compared with corn-based diets. Birds fed wheat/barley-based diets with 0.05% exogenous NSPases supplementation presented decreased FCR when compared with the wheat/barley-based control diet (Table 5).

Carcass characteristics

The economical characteristics of the carcass were not significantly affected by diet (p>0.05). Nevertheless, the best performance for the eviscerated ready-to-cook carcass was observed in birds fed corn-based diets supplemented with commercial enzyme mixtures (66%). Birds fed wheat/barley-based diet with 0.05% NSPases showed the highest relative weight of both breast (21%) and drumsticks (25%), which was equal to the results of birds fed corn-based

Table 4. Effects of supplementation of corn and a wheat/barley-based diets with enzyme mixtures (Kemin® and Rovabio®, at two dose levels each) on body weight gain (g/chick/period; mean ± sem).

Trait / Dietary treatment		Starter period (1st-14th d of age)	Grower period (15th-28th d of age)	Finisher period (29th-42nd d of age)	Total period (1st-42nd d of age)
Basal diet	Wheat-barely	245.841^{b} ± 6.277	776.616^{b} ± 18.701	1372.191^{b} ± 32.176	2396.900^{b} ± 55.208
	Corn	278.175^{a} ± 6.277	936.458^{a} ± 18.701	1668.633^{a} ± 32.176	2883.308^{a} ± 55.208
Enzyme type in diet	Kemin®	262.158^{a} ± 6.277	854.591^{a} ± 18.701	1516.475^{a} ± 32.176	2633.225^{a} ± 55.208
	Rovabio®	261.858^{a} ± 6.277	858.483^{a} ± 18.701	1524.350^{a} ± 32.1.76	2646.983^{a} ± 55.208
Enzyme level (% in diet)	0.025	246.158^{b} ± 6.277	53.770^{a} ± 18.701	1489.091^{a} ± 32.176	2564.141^{a} ± 55.208
	0.05	277.858^{a} ± 6.277	53.770^{a} ± 18.701	1551.733^{a} ± 32.176	2716.066^{a} ± 55.208
T1: Corn without enzyme		383.233^{a} ± 9.211	951.233^{a} ± 17.959	1681.000^{a} ± 30.109	3015.466^{a} ± 54.908
T2: Corn with Kemin® (0.025%)		281.333^{b} ± 9.211	931.000^{a} ± 17.959	1661.600^{a} ± 30.109	2873.933^{b} ± 54.908
T3: Corn with Kemin® (0.05%)		277.133^{b} ± 9.211	938.133^{a} ± 17.959	1671.066^{a} ± 30.109	2886.333^{b} ± 54.908
T4: Corn with Rovabio® (0.025%)		278.100^{b} ± 9.211	934.033^{a} ± 17.959	1668.300^{a} ± 30.109	2880.600^{b} ± 54.908
T5: Corn with Rovabio® (0.05%)		276.133^{b} ± 9.211	942.666^{a} ± 17.959	1673.566^{a} ± 30.109	2892.366^{b} ± 54.908
T6: Wheat-barely without enzyme		207.100^{c} ± 9.211	714.066^{c} ± 17.959	1304.066^{c} ± 30.109	2225.233^{e} ± 54.908
T7: Wheat-barely with Kemin® (0.025%)		211.166^{c} ± 9.211	718.233^{c} ± 17.959	1310.066^{c} ± 30.109	2239.466^{de} ± 54.908
T8: Wheat-barely with Kemin® (0.05%)		279.000^{b} ± 9.211	831.000^{b} ± 17.959	1423.166^{b} ± 30.109	2533.166^{c} ± 54.908
T9: Wheat-barely with Rovabio® (0.025%)		214.033^{c} ± 9.211	723.133^{c} ± 17.959	1316.400^{c} ± 30.109	2262.566^{d} ± 54.908
T10: Wheat-barely with Rovabio® (0.05%)		279.166^{b} ± 9.211	834.100^{b} ± 17.959	1439.133^{b} ± 30.109	2552.400^{c} ± 54.908

Growth period values (within columns) with different superscripts letters ($^{a, b, c, d, e, f}$) differ significantly (p<0.05).

diet without enzyme supplementation. Additionally, birds submitted to wheat/barley-based diets with 0.025 or 0.05% of Kemin® or Rovabio® mixtures showed the same relative weight of liver (3%) and bile (3%) than corn and wheat/barley-control diets. Regarding gizzard, birds submitted to wheat/barley-based diets with 0.025% Kemin® or Rovabio® presented the same relative weight as birds on the corn and wheat/barley-control diets (3%).

Blood parameters

The blood parameters determined in serum were not significantly affected by feed treatment except for GLU, VLDL and HDL (p>0.05; Tables 6 and 7). The highest and the lowest levels of serum GLU were observed in birds fed corn and wheat/barley-based diets without exogenous enzymes. Despite not significant (p>0.05), the inclusion of NSPases in corn and wheat/barley-based diets resulted, respectively, in low and high GLU mean values. Yet, serum GLU concentrations were lower in wheat/barley-based diets supplemented with 0.025 Kemin® or 0.025% Rovabio® (Table 6). In the groups submitted to these treatments, both serum VLDL and HDL levels did not differ from controls (p>0.05), while all other feed treatments resulted in significant decrease of VLDL and increase of HDL concentrations when compared with controls (p<0.05; Table 6). When data were analyzed independently of enzyme type and level, serum concentration of TC was higher in birds fed wheat/barley-based diets (p<0.05).

The BWG, FCR, and serum blood VLDL and HDL levels were clearly affected (p<0.05) by the interaction between basal diet and enzyme type (Table 8).

Discussion

Even though the diets were formulated to be isoenergetic and isonitrogenous, birds fed corn-based

Table 5. Effects of supplementation of corn and a wheat/barley-based diet with enzyme mixtures (Kemin® and Rovabio®, at two dose levels each) on feed conversion ratio (FCR; g/chick/period; mean ± sem).

Dietary treatment	Trait	Starter period (1st-14th d of age)	Grower period (15th-28th d of age)	Finisher period (29th-42nd d of age)	Total period (1st-42nd d of age)
Basal diet	Wheat-barely	1.481^{a} ± 0.029	1.733^{a} ± 0.028	2.123^{a} ± 0.034	1.929^{a} ± 0.025
	Corn	1.303^{b} ± 0.029	1.496^{b} ± 0.028	2.064^{a} ± 0.034	1.782^{b} ± 0.025
Enzyme type in diet	Kemin®	1.393^{a} ± 0.029	1.610^{a} ± 0.028	2.143^{a} ± 0.034	1.871^{a} ± 0.025
	Rovabio®	1.391^{a} ± 0.029	1.620^{a} ± 0.028	2.044^{a} ± 0.034	1.840^{a} ± 0.025
Enzyme level (% in diet)	0.025	1.468^{a} ± 0.029	1.658^{a} ± 0.028	2.185^{a} ± 0.034	1.920^{a} ± 0.025
	0.05	1.316^{b} ± 0.029	1.571^{a} ± 0.028	2.002^{b} ± 0.034	1.790^{b} ± 0.025
T1: Corn without enzyme		1.056^{c} ± 0.337	1.503^{d} ± 0.026	2.070^{abc} ± 0.028	1.763^{bc} ± 0.023
T2: Corn with Kemin® (0.025%)		1.310^{b} ± 0.337	1.680^{d} ± 0.026	2.223^{ab} ± 0.028	1.803^{bc} ± 0.023
T3: Corn with Kemin® (0.05%)		1.303^{b} ± 0.337	1.496^{d} ± 0.026	2.003^{abc} ± 0.028	1.773^{bc} ± 0.023
T4: Corn with Rovabio® (0.025%)		1.316^{b} ± 0.337	1.503^{d} ± 0.026	2.050^{abc} ± 0.028	1.800^{bc} ± 0.023
T5: Corn with Rovabio® (0.05%)		1.283^{b} ± 0.337	1.493^{d} ± 0.026	1.980^{bc} ± 0.028	1.735^{c} ± 0.023
T6: Wheat-barely without enzyme		1.620^{a} ± 0.337	1.813^{a} ± 0.026	2.243^{a} ± 0.028	2.050^{a} ± 0.023
T7: Wheat-barely with Kemin® (0.025%)		1.620^{a} ± 0.337	1.823^{a} ± 0.026	2.236^{a} ± 0.028	2.046^{a} ± 0.023
T8: Wheat-barely with Kemin® (0.05%)		1.340^{b} ± 0.337	1.616^{c} ± 0.026	2.110^{abc} ± 0.028	1.863^{b} ± 0.023
T9: Wheat-barely with Rovabio® (0.025%)		1.626^{a} ± 0.337	1.813^{a} ± 0.026	2.230^{a} ± 0.028	2.033^{a} ± 0.023
T10: Wheat-barely with Rovabio®(0.05%)		1.340^{b} ± 0.337	1.680^{b} ± 0.026	1.916^{c} ± 0.028	1.773^{bc} ± 0.023

Growth period values (within columns) with different superscripts letters ($^{a, b, c, d, e, f}$) differ significantly (p<0.05).

diet consumed more feed over the entire experiment, grew better and had a lower feed conversion ratio (FCR) compared with wheat/barley-based diets. It appeared that the FCR for birds fed corn-based diets could not be further improved by supplementation with NPSases while BWG and the FCR in birds fed wheat/barley-based diets can be significantly improved with the incorporation of 0.05% mixture of exogenous xylanases and glucanases. If 0.05% exogenous NSP-degrading enzymes are added to the wheat/barley-based diets, the final FCR were similar to those observed in birds fed corn. This result suggests that the digestibility of wheat/barley-based poultry diets can be improved by adding exogenous enzymes. Our results are consistent with previous findings (Mathlouthi *et al*., 2003a; 2003b; Shakouri *et al*., 2009). In a study conducted by Kocher *et al.* (2015), an enzyme product containing protease and xylanase activities was included in wheat-soybean meal diets fed to broilers from 1 to 42 d of age. The enzyme product improved average daily gain and feed conversion ratio, especially in birds up to 21 d of age. The simultaneous inclusions of phytase with α-galactosidases, protease, β-glucanase, and xylanase in corn-, barley-, or wheat-based broiler diets has additive effects in nutritionally marginal broiler diets. It appears that the activity of one type of feed enzyme may be facilitated by the other, possibly in a reciprocal fashion, by providing greater substrate access, and also by reducing the anti-nutritive effects of the substrates (NSP and phytate) on nutrient utilization (Ravindran, 2013b).

The magnitude of response in supplemented corn-based diets is generally lower than would be expected for diets based on wheat (Rosen, 2002). The main reason for this difference is the considerable different concentration of NSPs in wheat and barley compared with corn. Poor nutritional value of the latter two has been recognized for decades due to the presence

Table 6. Effects of supplementation of corn and a wheat/barley-based diets with enzyme mixtures (Kemin® and Rovabio®, at two dose levels each) on the blood parameters of broilers (mean ± sem).

Dietary treatment	Trait	Glucose (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	VLDL (very low density lipoprotein; mg/dL)	HDL Cholesterol (high density lipoproteins; mg/dL)
Basal diet	Wheat-barely	132.500^{b} ± 7.380	130.583^{a} ± 2.251	112.000^{a} ± 3.401	29.250^{a} ± 1.195	71.250^{b} ± 2.440
	Corn	181.000^{a} ± 7.380	125.750^{a} ± 2.251	95.000^{b} ± 3.401	23.000^{b} ± 1.195	84.666^{a} ± 2.440
Enzyme type in diet	Kemin®	153.500^{a} ± 7.380	127.750^{a} ± 2.251	103.250^{a} ± 3.401	26.250^{a} ± 1.195	78.166^{a} ± 2.440
	Rovabio®	160.000^{a} ± 7.380	128.583^{a} ± 2.251	103.750^{a} ± 3.401	26.000^{a} ± 1.195	77.750^{a} ± 2.440
Enzyme level (% in diet)	0.025	140.000^{b} ± 7.380	131.250^{a} ± 2.251	111.250^{a} ± 3.401	29.250^{a} ± 1.195	72.500^{b} ± 2.440
	0.05	173.500^{a} ± 7.380	125.083^{a} ± 2.251	95.750^{a} ± 3.401	23.000^{b} ± 1.195	83.416^{a} ± 2.440
T1: Corn without enzyme		239.000^{a} ± 9.676	140.000^{a} ± 2.817	130.000^{a} ± 4.124	35.000^{a} ± 1.212	60.000^{b} ± 2.408
T2: Corn with Kemin® (0.025%)		168.000^{bcd} ± 9.676	126.000^{a} ± 2.817	95.000^{a} ± 4.124	23.000^{b} ± 1.212	85.000^{a} ± 2.408
T3: Corn with Kemin® (0.05%)		185.000^{ab} ± 9.676	124.000^{a} ± 2.817	94.000^{a} ± 4.124	23.000^{b} ± 1.212	84.000^{a} ± 2.408
T4: Corn with Rovabio® (0.025%)		179.000^{abc} ± 9.676	127.000^{a} ± 2.817	96.000^{a} ± 4.124	24.000^{b} ± 1.212	83.000^{a} ± 2.408
T5: Corn with Rovabio® (0.05%)		192.000^{ab} ± 9.676	126.000^{a} ± 2.817	95.000^{a} ± 4.124	22.000^{b} ± 1 .212	86.000^{a} ± 2.408
T6: Wheat-barely without enzyme		96.000^{e} ± 9.676	138.000^{a} ± 2.817	128.000^{a} ± 4.124	36.000^{a} ± 1.212	59.000^{b} ± 2.408
T7: Wheat-barely with Kemin® (0.025%)		103.000^{de} ± 9.676	136.000^{a} ± 2.817	127.000^{a} ± 4.124	35.000^{a} ± 1.212	61.000^{b} ± 2.408
T8: Wheat-barely with Kemin® (0.05%)		158.000^{bcde} ± 9.676	125.000^{a} ± 2.817	97.000^{a} ± 4.124	24.000^{b} ± 1.212	82.000^{a} ± 2.408
T9: Wheat-barely with Rovabio® (0.025%)		110.000^{cde} ± 9.676	136.000^{a} ± 2.817	127.000^{a} ± 4.124	35.000^{a} ± 1.212	61.000^{b} ± 2.408
T10: Wheat-barely with Rovabio® (0.05%)		159.000^{bcde} ± 9.676	125.333^{a} ± 2.817	97.000^{a} ± 4.124	23.000^{b} ± 1.212	81.000^{a} ± 2.408

Growth period values (within columns) with different superscripts letters ($^{a, b, c, d, e, f}$) differ significantly (p<0.05).

of arabinoxylans in wheat and arabinoxylans and β-glucans in barley (Bedford, 1995; Saulnier *et al*., 1995). The mechanisms involved in poor digestibility are not fully understood but it is recognized that viscosity reduces the intestinal passage rate (van der Klis *et al*., 1993), reduces the diffusion of digestive enzymes and stimulates bacterial proliferation in the small intestine (Choct, 2006; Rodriguez *et al*., 2012). Moreover, the water-holding capacity brought about by soluble NSP markedly influenced voluntary feed intake. The breakdown of these NSPs via enzyme addition contributes to decrease viscosity of the gut contents (Bedford and Classen, 1992), improving feed intake and efficiency of nutrient utilization (Mathlouthi *et al*., 2002; Choct *et al*., 2004; Shakouri *et al*., 2009; Bedford and Partridge, 2010; Rodriguez *et al*., 2012).

Plasma GLU, protein and lipid levels reflect the rate of digestion. Starch is the major dietary carbohydrate in poultry diets. Fecal starch digestibility (% of dry matter) in cereal grains is almost total (Yutste *et al*., 1991), but the dynamics of starch digestion can affect plasma glucose levels, which may have consequences for protein utilization (Weurding *et al*., 2003). Despite differences between cultivars (Mollah *et al*., 1983; del Alamo *et al*., 2009), starch digestion coefficients of wheat (93.8%) are lower than those of barley (98.3%), and corn (97.4%; Weurding *et al*., 2001). In the results presented here, VLDL decreased and HDL increased

Table 7. Effects of supplementation of corn and a wheat/barley-based diets with enzyme mixtures (Kemin® and Rovabio®, at two dose levels each) on the blood parameters of broilers (mean ± sem).

Dietary treatment	Trait	Total protein (g/dL)	Albumin (g/dL)	Uric acid (mg/dL)	Alkaline phosphatase (U/dL)
Basal diet	Wheat-barely	$3.000^a \pm 0.269$	$0.666^a \pm 0.064$	$3.250^a \pm 0.269$	$277.500^a \pm 5.362$
	Corn	$3.000^a \pm 0.269$	$0.666^a \pm 0.064$	$2.750^a \pm 0.269$	$277.500^a \pm 5.362$
Enzyme type in diet	Kemin®	$3.000^a \pm 0.269$	$0.666^a \pm 0.064$	$3.000^a \pm 0.269$	$268.000^a \pm 5.362$
	Rovabio®	$3.000^a \pm 0.269$	$0.666^a \pm 0.064$	$3.000^a \pm 0.269$	$287.000^a \pm 5.362$
Enzyme level (% in diet)	0.025	$3.000^a \pm 0.269$	$0.666^a \pm 0.064$	$3.000^a \pm 0.269$	$278.750^a \pm 5.362$
	0.05	$3.000^a \pm 0.269$	$0.666^a \pm 0.064$	$3.000^a \pm 0.269$	$276.250^a \pm 5.362$
T1: Corn without enzyme		$3.000^a \pm 0.239$	$1.000^a \pm 0.074$	$4.000^a \pm 0.264$	$302.000^a \pm 6.375$
T2: Corn with Kemin® (0.025%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$3.000^a \pm 0.264$	$261.000^a \pm 6.375$
T3: Corn with Kemin® (0.05%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$3.000^a \pm 0.264$	$264.000^a \pm 6.375$
T4: Corn with Rovabio® (0.025%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$2.000^a \pm 0.264$	$304.000^a \pm 6.375$
T5: Corn with Rovabio® (0.05%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$3.000^a \pm 0.264$	$281.000^a \pm 6.375$
T6: Wheat-barely without enzyme		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$4.000^a \pm 0.264$	$271.000^a \pm 6.375$
T7: Wheat-barely with Kemin® (0.025%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$3.000^a \pm 0.264$	$260.000^a \pm 6.375$
T8: Wheat-barely with Kemin® (0.05%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$3.000^a \pm 0.264$	$287.000^a \pm 6.375$
T9: Wheat-barely with Rovabio® (0.025%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$4.000^a \pm 0.264$	$290.000^a \pm 6.375$
T10: Wheat-barely with Rovabio® (0.05%)		$3.000^a \pm 0.239$	$0.666^a \pm 0.074$	$3.000^a \pm 0.264$	$273.000^a \pm 6.375$

Growth period values (within columns) with different superscripts letters ($^{a, b, c, d, e, f}$) differ significantly (p<0.05).

Table 8. Effect of basal diet (corn or wheat/barley-based), enzyme type (Kemin® or Rovabio®), and enzyme level (0.025 or 0.05%) on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), glucose (GLU), very low density lipoprotein (VLDL), and high density lipoprotein (HDL).

Factor	P-value					
	FI (g/chick)	BWG (g/chick)	FCR	GLU (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
Basal diet	0.000	0.000	0.000	0.000	0.001	0.002
Enzyme type	0.608	0.000	0.000	0.037	0.000	0.002
Enzyme level	0.313	0.098	0.155	0.669	0.855	0.935
Basal diet * Enzyme type	0.261	0.000	0.000	0.232	0.001	0.004
Basal diet * Enzyme level	0.493	0.361	0.362	0.869	0.855	0.935
Enzyme type * Enzyme level	0.250	0.888	0.289	0.869	0.584	0.805
Basal diet * Enzyme type * Enzyme level	0.420	0.920	0.492	0.974	0.855	0.682

when 0.05% enzymes were included in the wheat/barley-based diet. Serum protein levels, on the other hand, were not affected. The variations observed in serum GLU concentrations found in birds fed corn-based diets supplemented with enzymes seems to be due to the lower FI in the finisher period.

Viscous NSP can modify bacterial activity decreasing fat digestibility (Smits and Annison, 1996). Protein digestibility is also altered by increased intestinal viscosity (Wang *et al*. 1992). In this study we could not find significant effects of diets and supplements on the lipid and protein profiles in plasma. Additionally, no statistical significant changes were found for TG, TC, TP, Alb, UAc, and ALP plasmatic concentrations between dietary treatments.

Rodrıguez *et al*. (2012) found that a wheat/barley-based diet supplemented with xylanase plus β-glucanase significantly improved the apparent digestibility of dietary fat in broiler chickens. In our work, the statistical changes found for blood VLDL and HDL profiles in birds fed enzyme-supplemented diets indicate that the absorption of these metabolites in the intestine is modulated by exogenous enzymes possibly in virtue of intestinal microflora.

It is commonly accepted that fat deposition is positively correlated with VLDL, HDL, and cholesterol blood levels, and that fat deposition depends mainly on available VLDL transported triglycerides. In our study, looking towards the overall changes in the serum VLDL and HDL concentrations in birds fed enzymes, it can be concluded that enzymes cause a lower availability of lipids for fat deposition, even though HLDL levels are higher than those of control diets. Since these kinetic parameters are nearly equal, it is possible that also abdominal fat deposition in the carcass was not different.

In this study, it was shown that body weight gain in birds fed wheat/barley-based diets can be improved by supplementation with 0.05% NSP enzyme mixtures (Kemin® and Rovabio®) such that birds reached the same FCR as birds fed supplemented and non-supplemented corn-based diets. Moreover, these birds maintained good carcass characteristics, supporting the use of exogenous NPSases enzymes in wheat/barley-based diets.

In conclusion, the results showed the beneficial effect of NSPase supplementation on diets based on a mixture of wheat and barley, rich in NSP and a high fiber content, which could translate into economic benefits.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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