

Inclusion of *Anastrepha ludens* fruit fly pupae in poultry rations added with digestive enzymes and yeast

Inclusión de pupas de mosca de la fruta *Anastrepha ludens* en raciones para aves adicionadas con enzimas digestivas y levadura

Inclusão de pupas de mosca-das-frutas *Anastrepha ludens* em rações avícolas com adição de enzimas digestivas e levedura

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Abstract

In Mexico, poultry meat represents 24,8 % of the protein consumed. In the search for protein sources that meet this demand, the use of insects has been found to be of potential interest. In order to reduce the effect of the components that affect the absorption of nutrients, additives such as digestive enzymes and microorganisms have been used. The objective of this study was to determine the weight gain of birds (*Gallus gallus domesticus*) fed with diets formulated with fruit fly pupa (*Anastrepha ludens*), digestive enzymes and yeast (*Saccharomyces cerevisiae*). Groups were established completely at random with different inclusions of fly pupae 0 %, 12 %, 14 % and 16 % respectively and significant differences were found ($p < 0.05$). The group with 14 % protein was the one with the greatest weight gain during the experiment and the second phase used digestive enzymes and *Saccharomyces cerevisiae* and it was found that treatment 3: 14 % of *Anastrepha ludens* pupa + 200 IU of Protease + 1502 IU of Amylase + 80 IU of Cellulase + 62 IU of Lipase + 40 IU of Pectinase + 8.88×10^9 CFU *S. cerevisiae* / 100 g of feed showed differences ($p < 0.05$) and the best results in the weight gain of the birds. It is concluded that inclusion of 14 % of fly pupa in rations promotes the weight gain of *Gallus gallus domesticus* with the addition of digestive enzymes and *S. cerevisiae*.

Resumen

En México la carne de aves representa el 24,8 % de la proteína consumida. En la búsqueda de fuentes proteicas que cubran esa demanda se han encontrado con interés potencial la utilización de insectos. Con la finalidad de reducir el efecto de los componentes que afectan la absorción de nutrientes se han utilizado aditivos como enzimas digestivas y microorganismos. El objetivo del presente estudio fue determinar la ganancia de peso de aves (*Gallus gallus domesticus*) alimentadas con dietas formuladas con pupa de mosca de la fruta (*Anastrepha ludens*), enzimas digestivas y levadura (*Saccharomyces cerevisiae*). Se establecieron grupos completamente al azar con diferentes inclusiones de pupa de mosca 0 %, 12 %, 14 % y 16 % respectivamente y se encontraron diferencias significativas ($p < 0,05$). El grupo con 14 % de proteína fue el que mayor ganancia de peso tuvo durante el experimento y la segunda fase se utilizaron enzimas digestivas y *S. cerevisiae* y se encontró que el tratamiento 3: 14 % de pupa de *Anastrepha ludens*+ 200 UI de Proteasa+1502 UI de Amilasa+ 80 UI de Celulasa+ 62 UI de Lipasa+ 40 UI de Pectinasa+ $8,88 \times 10^9$ UFC *S. cerevisiae* / 100 g de alimento mostró diferencias ($p < 0,05$) y los mejores resultados en la ganancia de peso de las aves. Se concluye que la inclusión de 14 % de pupa de mosca en raciones promueve para ganancia de peso de *Gallus gallus domesticus* con la adición de enzimas digestivas y *S. cerevisiae*.

Palabras clave: Alimentación, probióticos, proteasas, subproductos proteicos.

Resumo

No México, a carne de frango representa 24,8 % da proteína consumida. Na busca por fontes proteicas que atendam a essa demanda, o uso de insetos tem se mostrado de potencial interesse. Para reduzir o efeito dos componentes que afetam a absorção de nutrientes, aditivos como enzimas digestivas e microrganismos têm sido utilizados. O objetivo deste trabalho foi determinar o ganho de peso de aves (*Gallus gallus domesticus*) alimentadas com dietas formuladas com pupa de mosca-das-frutas (*Anastrepha ludens*), enzimas digestivas e levedura (*Saccharomyces cerevisiae*). Os grupos foram estabelecidos de forma completamente aleatória com diferentes inclusões de pupas de mosca 0 %, 12 %, 14 % e 16 %, respetivamente, e diferenças significativas foram encontradas ($p < 0,05$). O grupo com 14 % de proteína foi o que apresentou maior ganho de peso durante o experimento e a segunda fase utilizou enzimas digestivas e *Saccharomyces cerevisiae* e verificou-se que o tratamento 3: 14 % de *Anastrepha ludens* pupa + 200 UI de Protease + 1502 UI de Amilase + 80 UI de Celulase + 62 UI de Lipase + 40 UI de Pectinase + $8,88 \times 10^9$ UFC de *S. cerevisiae* / 100 g de ração apresentaram diferenças ($p < 0,05$) e os melhores resultados no ganho de peso das aves. Conclui-se que a inclusão de 14 % de pupa de mosca nas rações promove o ganho de peso de *Gallus gallus domesticus* com a adição de enzimas digestivas e *S. cerevisiae*.

Palavras-chave: Alimentos, probióticos, proteases, subprodutos de proteínas.

Introduction

The poultry industry is one of the most important livestock activities in Latin American countries (FAO, 2015). In Mexico, it participates with 24.8 % of animal protein consumed (UNA, 2015;

SAGARPA, 2015); particularly in rural areas, backyard poultry farming is the main source of animal protein (egg and meat) for human consumption (SAGARPA, 2007; SEDESOL, 2010).

In commercial poultry farming, soybean meal, meat and fish are the primary sources of protein (FAO, 2013), however, in backyard conditions, the feeding is made, primarily, based on grains (corn, sorghum) which on many occasions, do not cover the protein needs of the birds (Medina, 2012). Such deficiency limits the productive and reproductive levels (Sánchez, 2013). In addition, since corn is the base of the Mexican diet, a food competition is established for the same input (SAGARPA, 2015; USDA, 2015). On the other hand, cereal-based poultry rations contain non-starch polysaccharides (NAP) such as cellulose and hemicellulose, which affect the absorption of nutrients, causing deficiencies in animal nutrition and development (Choct *et al.*, 2010).

In the search for alternative sources of protein useful for animal feed and available in the environment, a large number of research works have been carried out (SEDESOL, 2010; FAO, 2015). In this sense, due to the quantity and quality of protein that insects have (Veldkamp *et al.*, 2012; Sancho *et al.*, 2015), they can be an alternative for feeding birds, with the advantage that birds naturally ingest adult insects, larvae, and pupae (Hwangbo *et al.*, 2009). These contain amounts of protein and fat comparable with commercial protein meal (Barroso *et al.*, 2014; Józefiak and Engberg, 2015), in addition, that the composition of chitin (exoskeleton) has been found to favor the immune response, the growth of beneficial bacteria and inhibits the growth of pathogenic microorganisms (Van Huis *et al.*, 2013).

Likewise, the use of biological additives (microorganisms and enzymes) in the animal diet improves the availability of nutrients (Kiarie, 2013), within these there are yeasts, mainly *Saccharomyces cerevisiae* that has been used in different species such as bovines, pigs and poultry as an inhibitor of pathogens such as *Salmonella* sp., digestion enhancers, promoting the action of digestive enzymes and stimulating immunity (Ogbuewu *et al.*, 2018). Likewise, they favor an increase in the size and number of intestinal villi, and maintain a better state of immunocompetence and prevent colonization of the intestine by *Salmonella* sp. (Hassanein and Soliman, 2010; Adebisi *et al.*, 2012, Kiros, *et al.*, 2019).

For their part, exogenous enzymes help to hydrolyze molecules facilitating the digestion of food and producing a better development by birds (Bedford and Patridge, 2001; Méndez *et al.*, 2009). Enzymes can be classified according to polygastric or monogastric species and in the latter case it is where the catalytic biomolecules that normally cannot be metabolized by birds, although due to their microbiota, have been used recently. They promote better digestion, improve intestinal transit and modify the consistency of the stool allowing greater digestibility of the nutrients. It has also been found that in young birds these enzymes promote a better productive performance of the same (Mulisa, 2017). Therefore, the objective of this work was to determine the weight gain of birds (*Gallus gallus domesticus*) fed with diets formulated with fruit fly pupa (*Anastrepha ludens*), digestive enzymes and yeast (*S. cerevisiae*).

Materials and methods

The work was carried out at the “Ayol” agroecological farm (14°49'45”N, 92°17'47” W) located in Tapachula, Chiapas and in the research Laboratorio de Investigación del Instituto de Biotecnología de la Universidad Autónoma de Chiapas (UNACH).

The experiment was carried out in two phases. Phase 1: To determine the effect of the fly pupa (*A. ludens*) and biological additives (digestive enzymes and *S. cerevisiae*) on the development of *Gallus gallus domesticus* fed with isoprotein rations designed for this purpose, the following experiment was established: Isoprotein rations were designed according to the nutritional requirements of the birds (NRC, 1994) with *A. ludens* pupa as a non-conventional protein source. The rations were made with corn, commercial kernel and fly pupa, whose proximal chemical content was analyzed by quantifying crude protein, crude fat, acid detergent fiber, moisture, ash and nitrogen-free extract (NMX-f-083- 1986, NMX-f-066-s-1978, NMX-f-089-s-1978, NOM-F-68-S-1980, NOM-F-90-S-1978.) (table 1).

Table 1. Proximal chemical analysis of ingredients (g.100g⁻¹).

Determination	Maice	Fly pupa	Core
Crude protein	8.9 ± 0.1	49.21 ± 0.3	19.78 ± 0.17
Crude fat	2.40 ± 0.21	1.48 ± 0.38	2.74 ± 0.1
Fiber	4.49 ± 0.37	0.0025 ± 0.005	4.94 ± 0.12
Ash	0.87 ± 0.14	8.53 ± 0.27	5.6 ± 0.16
NFE ¹	83.27 ± 0.2	40.85 ± 0.24	66.92 ± 0.13

Values on a dry basis. 1Nitrogen-free extract

To evaluate the weight gain of the birds fed these rations, 100 male individuals were assigned to treatments in 4 groups of birds of the Sussex line of one month of age and an average weight of 415 ± 0.1 g with 25 individuals each. In a group in a completely randomized design with 4 repetitions (one bird as an experimental unit and 24 more birds as a repetition), the weight gain and mortality rate were measured for a period of 30 days.

Table 2. Ingredients and nutritional composition in the final stage rations.

Ingredients %	Control treatment	Treatment 1	Treatment 2	Treatment 3
Fly pupa	0	12	14	16
Maice	64	68	74	79
Core	36	20	12	7
Valor nutricional calculado				
Protein	14.43	14.43	14.36	14.30
Fat	2.38	2.14	2.09	2.06
Fiber	4.34	3.67	3.55	3.44
Humidity	9.18	9.44	9.48	9.53
Ash	3.55	2.48	2.27	2.07
Energy ¹ (J)	1,106.58	1,135.37	1,141.89	1,148.09

¹Energy in joules.100 g⁻¹ of feed

Management: The birds were previously adapted to the rations with the gradual inclusion of the diet and immunized against enzootic diseases of the region (Pasteurellosis and Newcastle).

The first phase of the experiment concluded with the identification of the ration that promoted the greatest weight gain in the birds ($P < 0.05$) (table 3). This made it possible to know the degree of inclusion of the alternative protein source in isoprotein rations in order to design in a second phase rations with pupae of *A. ludens* added with different proportions of a biological additive: Protease 225 IU, Amylase 1690 IU, Cellulase 90 IU, Lipase 70 IU, Pectinase

45 IU; *S. cerevisiae* 1.0x10¹⁰ CFU per g of additive, and the weight gain and mortality rate were evaluated with a completely randomized design and 4x2 factorial arrangement (treatment and sex): T0: ration without biological additive, T1: 0.044 %; T2: 0.066 %; T3: 0.088 %, according to the nutritional requirements (table 4) of the birds during 69 days. For this, 80 female and male birds (*Gallus gallus domesticus*) one week old and an average weight of 94 ± 0.7 g were used, randomly distributed and included in each treatment (1 bird as an experimental unit and 19 birds as a repetition).

The statistical analysis consisted for the first and second phases of the experiment in an analysis of variance to determine the differences between the means of the treatments and a contrast of means by Tukey to identify the different treatment (s) with a confidence of 95 % ($P < 0.05$) with the statistical software Statgraphics Centurion XVI MR.

Results and discussion

The proximal chemical composition of the unconventional protein source (*A. ludens*) used for the preparation of isoprotein rations is shown in table 3. The proximal chemical composition of a wide variety of insects has been described in advance (Kouřimská and Adámková, 2016; Oonincx and Finke, 2020), but not the pupa of *A. ludens*, which so far lacks studies that describe its nutritional content. Pretorius (2011) found that the nutritional content of *Musca domestica* pupa is close to 60 % protein, which is higher than that found in the present work. On the other hand, Rumpold and Schlüter (2013), determined the nutritional composition of more than 200 insects and found that those of the order Diptera have a percentage range of 49.48 ± 13.61 of protein, lower in the reported fiber content 13.56 ± 3.81 and fat 22.75 ± 10.86; noting that *A. ludens* is a potential protein source to be used in domestic animal feed.

The weight gain of the birds between the treatments was different ($P \leq 0.05$) with the inclusion of 12 %, 14 % and 16 % of pupa in the diet and the control group (table 4).

The highest weight gain of the birds was obtained by including 14 % of *A. ludens* pupae in the diet with a mean gain of 857.30 ± 112.54 g during the evaluation period and a zero mortality rate.

Pretorius (2011) determined that with an inclusion of 10 % of *Musca domestica* in a conventional diet for birds, the best results are obtained in weight gain and efficiency in feed conversion, although the range extends to 25 % with favorable results. The inclusion of insects in the diet improved weight gain since according to Hwangbo *et al.* (2009) provides a positive effect due to their digestibility and nutritional value. According to De Marco *et al.* (2015) and Schiavone *et al.* (2017) the flour of *Tenebrio molitor* and *Hemeta illucens* respectively, are valuable sources of metabolizable energy and digestible amino acids that birds can take advantage of together with their rapid digestive process. Bovera *et al.* (2016) used flour from *Tenebrio molitor* in the larval stage and found that chickens develop better when insects are included as a source of protein, similar to this study. The source of fiber, the type and age of the birds influence the response of the birds to their diet (González *et al.* 2010; Oonincx and Finke, 2020).

The treatments evaluated with the inclusion of biological additives such as enzymes and yeasts in the diet prepared with 14 % of *A. ludens* (table 3) were different ($P \leq 0.05$). The treatment 3 with biological additive (200 IU of Proteases, 1502 IU of Amylases, 80 IU of Cellulase, 62 IU of Lipase, 40 IU of Pectinase, 8.88x10⁹ CFU

Table 3. Nutritional composition of the rations (g/100g^{1/3}) with the biological additive

Nutrient	Star	Development	End	T1	T2	T3
Protein	20.1	17	14.36			
Fat	2.27	2.19	2.09			
Fiber	3.80	3.67	3.55			
Moisture	9.19	9.35	9.48			
Ash	4.77	3.44	2.27			
AME ¹ (J)	1,001.98	1,076.96	1,141.89			
Enzymes²						
Protease				100	150	200
Amylase				751	1,127	1,502
Cellulase				40	60	80
Lipase				31	47	62
Pectinase				20	30	40
<i>S. cerevisiae</i> (CFU) ³				4,44x10 ⁹	6,66x10 ⁹	8,88x10 ⁹

¹AME. apparent metabolizable energy (Joules). ²Units of digestive enzymes. ³Colony-forming unit according to the Alka Rumen NRV commercial product information, NORVET^{MR} laboratories.

Table 4. ANOVA Weight gain of birds with isoprotein rations.

Groups	Weight gain (g)
Witness	757.67 ± 125.93 ^{bc}
T1	524.00 ± 71.06 ^c
T2	857.33 ± 112.54 ^a
T3	653.33 ± 140.79 ^{ab}

^{a, b, c} Different literals mean significant differences (p < 0.05).

of *S. cerevisiae*) had the greatest positive effect on the weight gain of birds, with an average of 1,902.09 g with respect to the control group that had 1,699.99 g of gain and the males had a significantly greater gain than that of the females (P ≤ 0.05) (table 5). However, the interaction between the additive and sex did not show differences (table 6 and 7), although Hristakieva *et al.* (2014) mention that the sex of the birds is decisive in their development as the males have a higher metabolic rate. For their part, enzymes and yeasts improved the digestibility of nutrients through hydrolytic processes, which birds are not capable of digesting (Carvajal and Oviedo, 2014), for example: non-starch polysaccharides (PNA) contained in corn. According to Choct *et al.* (2010), the use of enzymes for poultry neutralizes its effects in cereals, which are not desirable since they increase viscosity, reduce the digestion and absorption of all the nutrients in the diet, especially fat and protein.

On the other hand, Hajati (2010); Khan (2011); Kiarie *et al.* (2013), found that enzyme complexes promote weight gain and carcass performance, by improving the nutritional value and digestibility of poultry rations, that transform them into low molecular weight products that can pass directly through the intestinal mucosa or are absorbed; furthermore, Cho *et al.* (2012) observed that enzymes counteract the negative effects caused by the decrease in the concentration of nutrients.

Table 5. Phase two Experiment Weight gain of birds with biological additive (*Saccharomyces cerevisiae* + digestive enzymes) in the diet.

Source	P-Value	Tukey	Mean value
A: Treatment	0.0471	T3	1,902.09 ^a
		T2	1,777.12 ^{ab}
		T1	1,731.61 ^{ab}
		Witness	1,699.99 ^b
B: Sex	0.0000	Male	1,932.92 ^a
		Female	1,622.48 ^b
AB	0.6078		

No mortality was recorded during the biological additive inclusion phase (*Saccharomyces cerevisiae* + digestive enzymes), according to Guida *et al.* (2015) *S. cerevisiae* has a probiotic function in birds, since they observed that it can agglutinate pathogenic bacterial strains *in vitro*. This, attributed to the fact that naturally its wall formed by important natural growth promoters (β 1-3 glucan and mannan-proteins) are capable of inhibiting the colonization by pathogens of the digestive tract and adsorb mycotoxins (Zhang and Wang, 2008, Ogbuewu *et al.*., 2018). Shareef and Al-Dabbagh (2009) found that including up to 2 % of *Saccharomyces* in the diet was enough to achieve a competitive response in the production and mortality parameters, which coincides with that reported by Elghandour *et al.* (2019) who reported the use of yeast as a probiotic. The use of this yeast in the diet is directly related to the weight gain of the birds, it improves the intestinal mucosa, increases the intestinal villi, thereby increasing the activity of the enzymes secreted by the villi and absorption of nutrients (Arce *et al.*, 2008); however, the development of birds will be affected if the availability of nutrients is correct, Adebisi *et al.* (2012) found no differences in the weight of broilers using 1 %, 1.25 % and 1.5 %

yeast, although they presented morphological differences such as villus elongation, crypt depth and crypt gland area. in accordance with that described by Reyes *et al.* (2014) who observed that lower cell wall concentrations than 0.1 %, allows better development of organs in birds, increasing the absorption response.

Mortality of up to 6 % (Elghandour *et al.*, In 2019) has been reported in poultry fattening, although the parameters indicate that it should be less than 3 %. In the present work, the mortality rate was 0 % with the use of a biological additive that contained *Saccharomyces cerevisiae* + digestive enzymes in agreement with the previous author who mentions said yeast as a probiotic and improves the results.

Conclusion

The use of fly pupae (*Anastrepha ludens*) and biological additives (Proteases, Lipases, Cellulases, Pectinases, Amylases and *Saccharomyces cerevisiae*) in rations for poultry (*Gallus gallus domesticus*), promotes greater weight gain.

Mortality is not influenced by the use of the biological additives used in the experiment.

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