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Meta-analysis of the performance variation in broilers experimentally challenged by *Eimeria* spp.

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ABSTRACT

A meta-analysis was carried out to (1) study the relation of the variation in feed intake and weight gain in broilers infected with *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella*, or a Pool of *Eimeria* species, and (2) to identify and to quantify the effects involved in the infection. A database of articles addressing the experimental infection with Coccidia in broilers was developed. These publications must present results of animal performance (weight gain, feed intake, and feed conversion ratio). The database was composed by 69 publications, totalling around 44 thousand animals. Meta-analysis followed three sequential analyses: graphical, correlation, and variance-covariance. The feed intake of the groups challenged by *E. acervulina* and *E. tenella* did not differ ($P > 0.05$) to the control group. However, the feed intake in groups challenged by *E. maxima* and Pool showed an increase of 8% and 5% ($P < 0.05$) in relation to the control group. Challenged groups presented a decrease ($P < 0.05$) in weight gain compared with control groups. All challenged groups showed a reduction in weight gain, even when there was no reduction ($P < 0.05$) in feed intake (adjustment through variance-covariance analysis). The feed intake variation in broilers infected with *E. acervulina*, *E. maxima*, *E. tenella*, or Pool showed a quadratic ($P < 0.05$) influence over the variation in weight gain. In relation to the isolated effects, the challenges have an impact of less than 1% over the variance in feed intake and weight gain. However, the magnitude of the effects varied with *Eimeria* species, animal age, sex, and genetic line. In general the age effect is superior to the challenge effect, showing that age at the challenge is important to determine the impact of *Eimeria* infection.

1. Introduction

Coccidiosis is the most common and important parasitic disease in poultry industry. It is expected that all lots of chickens present infection by at least one species of *Eimeria* (Allen and Fetterer, 2002a). There are seven recognized species that affect the domestic chicken, but for broilers the main species are *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* due to high prevalence and substantial losses (Williams, 1999, 2005). The clinical coccidiosis with high mortality is not common, often occurring subclinical infection. So, it is often difficult to diagnose the disease at an appropriate period of time to begin a treatment before the animal start to show major losses on performance. This condition is accompanied by a reduction in feed intake. The low intake of nutrients is a factor that probably interferes in the weight gain (Duffy et al., 2005; Cornelissen et al., 2009). Besides interfering with feed intake, these protozoa can reduce the nutrients availability though lesions in the gastrointestinal tract and can also alter the animal metabolism

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by deviation of nutrients that would be used for growth, and thus compromising animal performance (Allen et al., 1998). For this reason, the feed given to sick animals, that is calculated based in the requirements of healthy animals, would be unbalanced and it would interfere in the expression of the maximum weight gain.

Most of the papers on performance of challenged broiler studied a specific infection (Conway et al., 1999; Kawazoe et al., 2005), or a product as an alternative to control the coccidiosis damage (Jang et al., 2007; Hassan et al., 2008; Wang et al., 2008; Peek et al., 2009), or a vaccine or immunization (Du et al., 2005; Lee et al., 2009; Shah et al., 2010a). So, there is a large diversity of experimental conditions and results observed in previous studies, this condition hinders to a global analysis of the variables involved. However, the relation between the reduction in feed intake and the alteration in weight gain is poorly understood. For this reason, this meta-analysis was carried out to study the relation of the variation in feed intake and in weight gain in broilers infected with *E. acervulina*, *E. maxima*, *E. tenella*, or a Pool of *Eimeria* species, identifying and quantifying the main effects involved in the infection.

2. Material and methods

2.1. Selection of articles and preparation of the database

Indexed publications with experimental infection with Coccidia in broilers were selected. Different online data sources (Scielo, Science Direct, High Wire, Google Scholar, and Scopus) were searched with keywords in several languages (English, Portuguese, Spanish, French, and Italian). The Coccidia species selected for this study were *E. acervulina*, *E. maxima*, *E. tenella*, and Pool (simultaneous infection with the three species).

The studies, after being identified, were critically evaluated for their quality and relevance in relation to the objectives of the meta-analysis. In this step, a set of information about each selected study was analyzed, including items related to the design, treatments, studied variables, and data analysis. After that, the selected trials were submitted to a checklist in order to evaluate their inclusion in this study. The main criteria for the publication selection were: (a) experimental infection with Coccidia in broilers, and (b) performance variables (feed intake and weight gain). The selection was performed independently by two evaluators based on the above criteria. Only data from studies published in indexed journals were selected, considering the acceptance for publication as a subjective criterion for their methodological quality. The results (positive or negative effect) were not taken as a selection criterion for inclusion of articles in the database. After the selection of the articles and the subsequent exploratory analysis, the information relative to the proposed objectives (animal performance) and other variables (genetic line, sex, nutritional composition of the diet, mortality index, production indexes, and injury score) were tabulated, in order to permit a descriptive analysis of the studies included in the database. The database was developed with information obtained from sections of material and methods and results of the selected articles. Each horizontal line in the database represented the summary of the characterization/results of one treatment/phase in the original study (selected papers) and each column denoted a variable of class (obtained in the material and methods section) or response (obtained in the result section).

2.2. Database description

The database was composed by 69 publications, totalling around 44 thousand animals distributed in 1085 treatments (Table 1). The average number of animals per treatment was 45 (ranging from 5 to 704). Regarding the categorization, there were 424 treatments with control animals, 236 treatments with animals challenged by *E. acervulina*, 139 by *E. maxima*, 163 by *E. tenella* and 123 treatments challenged by Pool in the database. Fifty-six treatments used females, 761 used males and 127 used mixed lots. The average age of the birds was 19 days (ranging from 1 to 59).

2.3. Encodings

The methodology used for data encoding followed the proposals described in the literature (Lovatto et al., 2007). The encodings were used for (1) classification (qualitative grouping criterion, or (2) adjustments in the variance-covariance analysis (moderating variables). The encodings with qualitative grouping criterion were used such as resources for associating homogeneous groups in certain characteristics and including them in the analytical models as a variation source. The encodings were used in statistical analysis as classes. In this particular, the main encodings used were for challenge (challenged or unchallenged animals). Another encoding was inserted to classify the experimental groups, being the treatments divided into five groups: control (unchallenged animals), challenged by *E. acervulina*, by *E. maxima*, by *E. tenella*, or by Pool. Other encodings were used as moderating variables in the analysis with the purpose of considering the variability of compiled studies (article, *inter*, and *intra* effects). For encoding the article effect (or general effect), specific sequential numbers were used for each study inserted in the database. The *inter* encoding was formed by uniting the general encoding with sequential numbers in such a way to give a specific code for each different treatment within the database. The *intra* encoding, similar to previous one, was attributed to each group with repeated measurements (in relation to body weight or age).

2.4. Graphic analysis

The meta-analysis followed three sequential analyses: graphical, correlation, and variance–covariance. The first analysis performed in the database was the graphical study. The main objective of this preliminary checking analysis was to verify the biological coherence of data through observations in scatter plots of each performance variable (feed intake, weight gain, and feed conversion ratio) *vs.* age, body weight, and calculated nutrient intake. Besides the observation of the data consistence, the graphic analysis was also important (1) to give an initial idea of

Table 1
Brief description of the database used in the meta-analysis of the performance variation in broilers.

Authors	Sex	Facilities	Species
Abbas et al. (2008)	–	Box	*E. tenella*
Alfaro et al. (2007)	Male	Cage	*Pool*
Allen and Fetterer (2002b)	Male	Cage	*E. maxima*
Allen and Fetterer (2002c)	Male	Cage	*E. maxima*
Allen et al. (1997)	–	Box	*E. maxima* and *E. tenella*
Augustine et al. (1997)	Male	Cage	*Pool*
Bafundo et al. (1984)	Male	–	*Pool*
Bafundo et al. (2008)	Male	Box	*Pool*
Banfield et al. (2002)	Female	–	*E. acervulina*
Bera et al. (2010)	–	–	*E. tenella*
Berezin et al. (2010)	–	Box	*E. tenella*
Biavatti et al. (2003)	Male	Box	*E. acervulina*
Brown and Southern (1985)	Male	Cage	*E. acervulina*
Conway et al. (1999)	Male	Cage	*E. acervulina*, *E. maxima* and *E. tenella*
Conway et al. (2001)	Mixed	Box	*Pool*
Conway et al. (2002)	–	Box	*E. tenella*
Cornelissen et al. (2009)	Male	Cage	*E. acervulina*, *E. maxima*, *E. tenella* and *Pool*
Czarnecki and Baker (1982)	Male	Cage	*E. acervulina*
Du et al. (2005)	–	Cage	*E. tenella*
Elmusharaf et al. (2007a)	Female	Cage	*Pool*
Elmusharaf et al. (2007b)	Male	Cage	*Pool*
Estrada et al. (2007)	Mixed	Box	*Pool*
Gabriel et al. (2003)	Male	Cage	*E. acervulina*, *E. maxima* and *E. tenella*
Gabriel et al. (2006)	Male	Box	*E. acervulina*, *E. maxima* and *E. tenella*
Giraldo et al. (1987)	Male	Cage	*E. acervulina*
Girgis et al. (2008)	Female	Cage	*Pool*
Gray et al. (1998)	Male	–	*E. acervulina*
Guo et al. (2007)	Mixed	–	*E. tenella*
Hassan et al. (2008)	Mixed	Cage	*E. tenella*
Hu et al. (2000)	Male	Cage	*Pool*
Jang et al. (2007)	Male	Cage	*E. maxima*
Jang et al. (2010a)	–	Cage	*E. acervulina*
Jang et al. (2010b)	–	Cage	*E. maxima*
Kidd et al. (2003)	Male	Box	*E. acervulina*
Klasing et al. (2002)	Mixed	Box	*E. acervulina*
Korver et al. (1997)	–	Box	*E. tenella*
Koynarski et al. (2007)	–	Cage	*E. acervulina*
Lee et al. (2007a)	–	Cage	*E. acervulina* and *E. tenella*
Lee et al. (2007b)	–	Box	*E. acervulina*
Lee et al. (2009)	–	Cage	*E. maxima* and *E. tenella*
Li et al. (2005)	Male	Cage	*Pool*
Mathis et al. (2003)	Mixed	Box	*Pool*
Matthews and Southern (2000)	Male	Box	*E. acervulina*
Matthews et al. (1997)	Male	Box	*E. acervulina*
Nollet et al. (2007)	Male	Box	*Pool*
Nweze and Obiwulu (2009)	–	–	*E. tenella*
Parker et al. (2007)	Male	Cage	*Pool*
Peek et al. (2009)	Male	Cage	*E. acervulina*, *E. maxima* and *E. tenella*
Persia et al. (2006)	Male	–	*E. acervulina*
Shah et al. (2010a)	–	Box	*E. tenella*
Shah et al. (2010b)	–	Cage	*E. acervulina*
Song et al. (2009)	–	–	*E. acervulina*, *E. maxima* and *E. tenella*
Southern and Baker (1982a)	Male	–	*E. acervulina*
Southern and Baker (1982b)	Male	–	*E. acervulina*
Southern and Baker (1982c)	Male	–	*E. acervulina*
Southern and Baker (1983a)	Male	–	*E. acervulina*
Southern and Baker (1983b)	Male	–	*E. acervulina*
Stanley et al. (2004)	Male	Box	*Pool*
Sun et al. (2005)	–	Box	*Pool*
Swinkels et al. (2006)	–	Cage	*E. acervulina*
Tamasaukas et al. (1999)	Mixed	Cage	*Pool*
Waldenstedt et al. (1999)	Female	Box	*Pool*
Wang et al. (2008)	Mixed	–	*E. tenella*
Watson et al. (2005)	Male	–	*E. acervulina*
Yadav and Gupta (2001)	–	Cage	*E. tenella*
Yi et al. (2005)	Mixed	Cage	*E. maxima*
Yim et al. (2011)	Male	Cage	*E. maxima*
Zhu et al. (2000)	Male	–	*E. maxima*
Zulpo et al. (2000)	–	Cage	*E. acervulina*, *E. maxima* and *E. tenella*

heterogeneity, (2) to observe the relation *intra* and *inter* studies, (3) and to identify the existence of interactions or non-linearity patterns (Lovatto et al., 2007).

2.5. Relation between variations in average daily feed intake and average daily weight gain

For each article inserted into the database, the infected groups were related with their respective control groups. The difference between the groups was expressed as percentage of variation in the average daily feed intake (ΔADFI) and percentage of variation in the average daily weight gain (ΔADG). These calculated variables, here analyzed using the General Linear Model procedure, considering the ΔADG as the response and the ΔADFI as the predictor. The model also include a moderating variable (encodings for article, *inter*, or *intra* effects).

2.6. Correlation and variance-covariance analysis

Spearman correlation test was performed between the diverse variables into the database. The criterion for defining the factors with high correlation were (1) to present correlation higher than 0.5, and (2) to be statistically significant ($P < 0.05$). The factors with high correlation with the response (feed intake and weight gain) in this previous test were used as covariable in the variance–covariance analysis. Based on the criterion previously established, the considered factors were: effect of animal age on feed intake; and effects of age and ΔADFI on weight gain.

A weighting scheme considering the number of repetitions in each measure was tested in analysis of variance. In this procedure, was tried to give more importance for studies which used more animals. However, the use of weighting for sample size did not influence the coefficient of determination in most of the analysis. Best coefficients of determination were obtained with the use of encodings in the models. This may be related with the information provided by Sauvant et al. (2008), that the importance of weighting procedure decreases with the number of observations used in the analysis.

General Linear Model procedure was used to perform the variance–covariance analysis (to compare the groups and also to obtain the prediction equations) and the subsequent variance decomposition (used to observe the intensity of model's variables on the variables under analysis). Variance decomposition was done using the *Sum of square* from analysis of variance. In the procedure, the total *Sum of square* was considered as 100% of total variance and the *Sum of square* of each variable was considered as a fraction of total variance. All variance–covariance analyses were performed considering treatments (− or + for challenge); the codification for general, *inter*, or *intra* effects; the sex (males, females, or mixed groups); and the genetic line. After this exploratory analysis, sex and genetic line were maintained in the models only when statistical significance ($P > 0.05$) was found for their effects. The factors that showed high correlation with the response in the previous test were used as covariable in the variance analysis. Based on these criterion, it was considered into the model of feed intake: the encoding for the challenge (+ or −, considering infected *vs.* control groups) and the encoding for general effect; and the age was used as covariable. The procedure was done individually for each infected group (*E. acervulina*, *E. maxima*, *E. tenella*, and Pool). For weight gain, the effects considered into the model were: the encoding for the challenge (+ or −, considering infected *vs.* control groups), the encoding for general effect, the sex and the genetic line; and the feed intake and the age were used as covariables.

The comparison of the adjusted means for feed intake, weight gain and feed conversion in relation to the treatment groups was performed by variance–covariance analysis, with subsequent comparison of means by Tukey test at 5% significance level. For the adjustment of means, the same variables used in the effects analysis were included in the models, with the exception of the challenge (+ or −) which was replaced by the encoding of sanitary group (control, *E. acervulina*, *E. maxima*, *E. tenella*, and Pool). All statistical analysis were performed using Minitab 15 Statistical Software (Minitab Inc., USA).

3. Results

3.1. Adjusted means

The performance variables are presented in Table 2. The means were adjusted for age, and the values presented in the table are representative for broilers with 18 days old, with the infection occurring on average at 13th day. The feed intake of the groups challenged by *E. acervulina* and *E. tenella* did not differ ($P > 0.05$) from the control group. The groups challenged by *E. maxima* and Pool increased the feed intake by 8% and by 5% ($P < 0.05$) compared with the control group.

All challenged groups decreased ($P < 0.05$) the weight gain when compared with control treatment. Challenged animals presented daily weight gain on average 10% lower than unchallenged ones, excepting the group challenged by *E. maxima* that showed a reduction of 23% compared with control group. The increase in feed intake and the large reduction in weight gain observed in the animals challenged by *E. maxima* resulted in the worsening in feed conversion ratio (140% higher than the control). The feed conversion ratio of the groups challenged by *E. acervulina*, *E. tenella*, and Pool were 13%, 49%, and 9% higher, respectively, when compared with the control group.

3.2. Identification and quantification of the effects

Through variance decomposition (Fig. 1), it was possible to identify the intensity of the effects on performance variables. Male and mixed groups were more resistant ($P < 0.05$) to *Eimeria* infection than the female groups.

Another factor that may interfere in the performance of challenged groups is the age of the broilers. The correlation between average age and feed intake of groups challenged by *E. acervulina*, *E. maxima*, *E. tenella* and Pool were 0.81, 0.82, 0.79, and 0.81, respectively. In the same way, the correlation between average age and weight gain of groups challenged by *E. acervulina*, *E. maxima*, *E. tenella*, and Pool were 0.63, 0.51, 0.54, and 0.55, respectively. In addition, the

Table 2
Adjusted means of feed intake, weight gain, and feed conversion ratio according to the treatment groups of broilers experimentally infected by *Eimeria* spp.*..

Variable	Control	*E. acervulina*	*E. maxima*	*E. tenella*	Pool	RSD
FI[1]	66.77^{b}	57.08^{b}	72.12^{a}	55.33^{b}	69.96^{a}	9.50
WG[2]	43.61^{a}	40.13^{b}	33.60^{c}	38.38^{bc}	39.29^{bc}	8.66
FCR[1]	1.57^{b}	1.77^{b}	3.78^{a}	2.33^{ab}	1.71^{b}	1.12

FI: feed intake, $g\,d^{-1}$; WG: weight gain, $g\,d^{-1}$; FCR: feed conversion ratio; RSD: residual standard deviation; 1: adjusted for the study effect and average age; 2: adjusted for the study effect, sex, genetic line, feed intake, and average age.
* Values with different letters in the same row differ ($P < 0.001$) by Tukey Test.

correlation between feed intake and weight gain of groups challenged by *E. acervulina*, *E. maxima*, *E. tenella*, and Pool were 0.96, 0.94, 0.95, and 0.96, respectively.

3.3. Relation between ΔADG and ΔADFI

The relation between ΔADG and ΔADFI is presented in Fig. 2. Through the observation of the intercepts of equations, it is possible to infer that the four challenged groups presented reduction in weight gain, even when there was no reduction in feed intake (zero % of variation in feed intake). Moreover, all curves presented a quadratic response.

4. Discussion

E. acervulina usually has been slightly pathogenic at an initial infection and its pathogenicity is dependent on the number of ingested oocysts (Graat et al., 1996; Kawazoe et al., 2005). So, its importance is greater in cases of massive environmental infection with recurrent reinfection (Gabriel et al., 2006; Assis et al., 2010). However, in infections which caused more than 20% of reduction in ΔADFI (Fig. 2), *E. acervulina* tended to cause more effect in ΔADG in relation with other species of *Eimeria*. It probably occurred because *E. acervulina* infects the duodenum, intestinal part with great importance in the absorption of

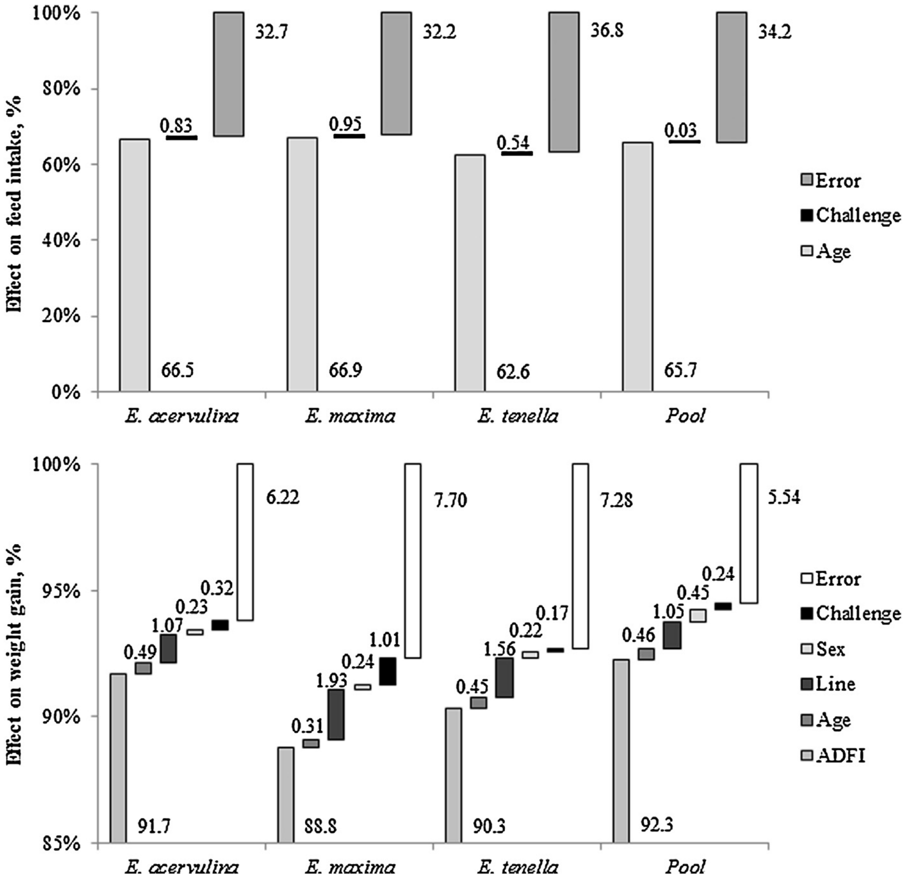

Fig. 1. Variance decomposition of feed intake and weight gain in broilers experimentally infected by *Eimeria* spp.

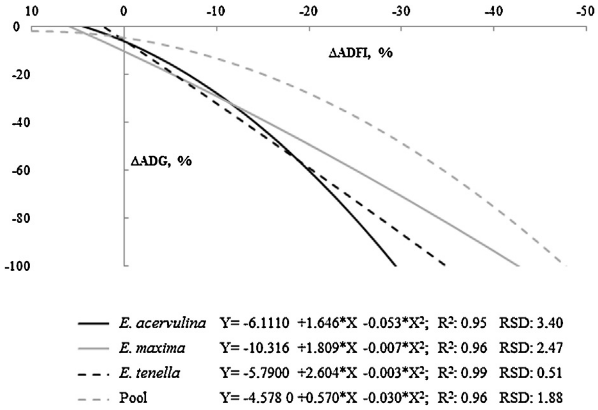

Fig. 2. Relation between the variation in average daily weight gain (ΔADG) due to the variation in average daily feed intake (ΔADFI) in broilers experimentally infected with *Eimeria* spp. RSD: residual standard deviation.

nutrients. So, in infections that caused little alteration in ΔADFI, *E. acervulina* tended to reduce the weight gain with similar intensity compared to other species, but when the negative effect on ΔADFI increased, *E. acervulina* tended to cause effects on the ΔADG in higher intensity.

E. maxima infect the middle section of the small intestine causing injuries from the duodenum to the ileum (Lillehoj and Trout, 1993). The extensive infected area in challenged animals may be related to the worst feed conversion ratio presented by animals infected with *E. maxima* (Idris et al., 1997). Analyzing the relation between ΔADG and ΔADFI, it is possible to observe that when there was no alteration in the ΔADFI, *E. maxima* caused the largest reduction in ΔADG compared with the other species of *Eimeria* but presenting less inclination in the curve (Fig. 2). It suggests that *E. maxima* has an important impact on ΔADG, but its relation is less pronounced when the reduction effect on ΔADFI increase.

In relation to the results *E. tenella* challenged groups, the age of the birds on challenge (average of 18 days old, with infection on average at 13 days old) may help to explain the similarity in feed intake of animals infected by *E. tenella* and the control group. *E. tenella* preferentially infects the cecum and adjacent intestinal regions, usually manifesting clinically with greater intensity after the fifth day of infection (Long and Joyner, 1984; Johnston et al., 2001). So, considering the average period presented in the papers used in the database, the broilers did not have enough time to express the main effects caused by the protozoan, such signs as reduction in weight gain and feed conversion ratio. Moreover, the cecal coccidiosis causes little nutritional effect and with short duration, but with severe clinical signs and consequent high mortality (Jeurissen et al., 1996; Mateos et al., 2002).

The variable with major effect on feed intake variance in challenged animals was the age (Fig. 1). Another variable that influenced in the weight gain variance was the animal sex. Females were more sensitive to the challenge effect compared to males or mixed lots, the last two groups were similar, corroborating with previous studies (Zhu et al., 2000). Another measured effect was the genetic line effect. In general, cross-breed animals were more resistant to coccidiosis effects compared to pure lines animals.

Comparing the effects caused by the challenges, the damage caused by *E. maxima* had the greatest influence in the weight gain. These results showed the complexity of factors that may act modulating the productive performance in challenged animals. Other factors, like environmental temperature and humidity, could also affect the results, but the analysis of environmental conditions was not possible because these data were poorly described in the papers used in the database.

The intercepts of ΔADG equations were negative (Fig. 2), indicating that infected animals showed weight gain reduced, even before the variation in feed intake. So, at constant feed intake, the weight gain was 6.1% lower in animals infected by *E. acervulina*, 10% lower in *E. maxima* challenge, 5.8% lower in *E. tenella* challenge, and 4.6% lower in the group infected by Pool compared to the uninfected broilers. So, infected animals could present weight gain similar to control group if they increase the feed/nutrient intake compared with the control group. The requested increase in feed intake would be around 4.3% for broiler infected by *E. acervulina*, 5.9% for *E. maxima*, and 2.2% for *E. tenella*. The same relation could not be observed for animals challenged by Pool because the equation for this group did not cross the ΔADG axis. However, the smallest loss in weight gain occurs when the animals consumed 9.5% more feed than uninfected ones. The increase in feed/nutrient intake of challenged animals has been mentioned as an alternative to reduce the deleterious effect caused by sanitary challenge on the performance of chickens (Klasing et al., 1987; Mateos et al., 2002; Andretta et al., 2011) and pigs (Kipper et al., 2011).

If we take into account that the feed conversion ratio is usually calculated by dividing feed intake in a period by weight gain in the same period, so Fig. 2 can be interpreted as the behavior of feed conversion ratio. When the data was analyzed at a fixed point of the *X* axis (ΔADFI) the farther point in relation to axis will be the worst FCR, so in the point zero of the *X* axis the group with best feed conversion ratio was the group challenged by Pool, followed by the groups challenged by *E. tenella*, *E. acervulina*, and *E. maxima*. On the other hand, when the data was analyzed at a fixed point on *Y* axis (ΔADG) the farther point in this axis will be the best FCR. So in the −100 point on *Y* axis, the group with best feed conversion ratio was the group challenged by Pool, followed by the groups challenged by *E. maxima*, *E. tenella*, and *E. acervulina*. In general, the FCR behavior is linear (Rosário et al., 2007). However, the highest coefficients of determination (R^2) were found in quadratic equations. This probably occurred due to the diversion of nutrients to the immune system and regeneration of injured tissues (Klasing et al., 1987).

5. Conclusions

The variation in feed intake of broilers infected by *E. acervulina*, *E. maxima*, *E. tenella*, and Pool is quadratic influenced by the variation of weight gain. Disconsidering the variation in feed intake, the more pathogenic species were

E. maxima, *E. acervulina*, *E. tenella*, and Pool. In general, the age effect is superior to the challenge effect, showing that age at the challenge is important to determine the impact of *Eimeria* infection.

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