

Qualitative feed restriction on productive performance and lipid metabolism in broiler chickens

[Restrição alimentar qualitativa sobre o desempenho produtivo e o metabolismo lipídico em frango de corte]

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ABSTRACT

A trial was carried out to evaluate the effect of qualitative restriction at different rearing phases on liver fatness and plasma triglycerides without impairing the productive performance of broiler chickens. One hundred and seventy-six male chicks were randomly assigned to four treatments and reared in battery brooders (16 pens with 11 birds each) from 1 to 49-day-old. In the control group, chicks were fed a starter diet (1 to 28-day-old) with 3,300kcal ME/kg of diet and 22.5% CP; grower diet (29 to 42-day-old) 3,300kcal ME/kg of diet and 18.2% CP; and the finisher diet (43 to 49-day-old) 3,300kcal ME/kg and 18.2% CP. Three restrict groups were fed a low 2,800kcal ME/kg of diet and 24% CP from 7 to 14-day-old (treatment 1); from 7 to 21-day-old (treatment 2); and from 22 to 35-day-old (treatment 3). All treatments were fed control diet before and after the feed restriction period. Feed intake was not affected during the feed restriction period; nevertheless, weight gain was lower ($P<0.05$) for chicks fed restricted diet from 7 to 21 and from 21 to 35-day-old. At the end of each restriction period, scores of liver for fatty infiltration were higher in restricted birds compared to control birds, although no changes were detected in plasma triglyceride levels. On the 49th day of age, less intracellular fatty infiltration was observed in the liver of previously restricted birds compared to control birds, and weight gain was also lower in the restricted groups. Moreover, triglyceride plasma levels were similar among treatments, but decreased in 12-hour-fasted broilers compared to non-fasted birds. The low energy:protein ratio diet affected weight gain and fatty infiltration in the liver, but had no effect on plasma levels of triglycerides.

Keywords: broiler chicken, feed restriction, productive performance, liver, triglyceride

RESUMO

Aplicou-se restrição alimentar qualitativa em frangos de corte, em diferentes fases do crescimento, para reduzir a infiltração gordurosa do fígado e os triglicerídeos plasmáticos, sem influir nas respostas produtivas. Distribuíram-se aleatoriamente 176 pintos machos em quatro tratamentos. As aves foram alojadas em baterias (16 gaiolas com 11 aves cada) e foram criados de 1 a 49 dias de idade. No grupo controle as aves foram alimentadas com dieta inicial (1-28 dias) com 3.300kcal EM/kg/dieta e 22,5% de proteína bruta (PB); dieta de crescimento (29-42 dias) com 3.300kcal EM/kg/dieta e 20,9% de PB; dieta de acabamento (43-49 dias de idade) 3.300kcal EM/kg/dieta e 18,2% de PB. As aves nos três grupos sob restrição foram alimentadas com dieta com 2.800kcal de EM/kg/dieta e 24% de PB de 7 aos 14 dias de idade (tratamento 1), de 7 aos 21 dias de idade (tratamento 2) e de 22 aos 35 dias de idade (tratamento 3). Todas as aves dos tratamentos sob restrição foram alimentadas com a dieta controle antes e depois do período de restrição alimentar. Ao final de cada período de restrição foi observada uma maior infiltração gordurosa no fígado (representado pela composição química e escore histológico). Também não houve diferenças entre os triglicerídeos plasmáticos, quando comparado com o controle. Aos 49 dias

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de idade, o ganho de peso total e a infiltração gordurosa no fígado, foram menores nos grupos restritos. Os níveis de triglicérides plasmáticos, entretanto, foram semelhantes entre os tratamentos, mas reduziram nas aves com aplicação de jejum de 12 horas, quando comparados com aves sem jejum. A baixa relação energia/proteína da dieta afetou o ganho de peso e a infiltração gordurosa no fígado, mas não alterou os valores de triglicérides plasmáticos.

Palavras-chave: frango de corte, restrição alimentar, resposta produtiva, fígado, triglicéride

INTRODUCTION

The incidence of heart diseases have increased in humans in the past years and has been associated to high fat consumption, especially saturated fat. Consequently, the consumption of meat containing lower levels of saturated fat (white meat), such as poultry, has increased. Qualitative and/or quantitative feed restriction of broilers might reduce the amount of fat or abdominal fat in carcasses. Qualitative restriction is related to nutrient dilution in the diet, whereas quantitative restriction to limiting the amount of feed daily given to the animals (Pokniak and Cornejo, 1982; Leeson and Zubair 1997).

Qualitative feed restriction is performed using diets with low energy to protein ratio (EPR); this is accomplished when dietary protein is increased or dietary energy is decreased or kept constant. As a consequence, the relative excess of protein induces higher energy utilization and, therefore, more synthesis and excretion of uric acid (Bartov, 1979). Unsaturated fatty acids (Clarke et al., 1990) or some drugs, such as beta agonists (Dalrymple et al., 1984), have been evaluated as a means to reduce fat levels in carcass, but the results are inconsistent. Quantitative restriction has been used for different purposes, such as reduction of broiler mortality and reduction of long bone abnormalities (Gonzales et al., 1998; Pelicano et al., 2005). Carcass fatness has decreased without impairment of body weight gain in broilers, but conflicting results have also been reported (Cornejo, et al., 1991; Furlan et al., 2002). Mathematic models have shown that the maximum abdominal fat weight is observed in 43-day-old broilers, whereas maximum abdominal fat gain expressed as carcass and live weight percentages occurs on the 15th and 19th day of age, respectively (Tzeng and Becker, 1981).

In poultry, the major site of “de novo” fat synthesis is the liver (Saadoun and Leclercq,

1987). Griffin et al. (1992) observed that the accumulated fat originated from plasma triglycerides, which in turn derived from the diet or were synthesized in the liver. Plasma triglycerides are detected as very low or low-density lipoprotein (VLDL and LDL, Griffin et al., 1982). According to Griffin and Whitehead (1982), VLDL-derived triglycerides are more available for fatty acid synthesis. In broilers, the levels of VLDL and LDL are correlated to fat deposition in the carcass. A twelve-hour fasting has been reported to reduce triglyceride plasma levels (Nir et al., 1984), and less liver lipogenesis has been observed in broilers that were fed diets containing higher proportions of energy derived from protein (Tanaka et al., 1983a). Based on these previous observations, an experiment was carried out to evaluate the effect of qualitative feed restriction on the performance, liver histology, and some variables of lipid metabolism in male broiler chickens.

MATERIAL AND METHODS

One hundred and seventy-six Hubbard broiler chicks were randomly assigned to four treatments in four replicates with 11 chicks each. The birds were reared from 1 to 28-day-age in battery brooders in an environmentally controlled room. On the 29th day of age, birds were transferred to batteries and reared until 49-day-old. During the first week, temperature was set at 33°C, and then it was decreased 3°C per week until 24°C. A 10-hours/day artificial light program was used during all the experiment. Water and feed were given *ad libitum*. The control group was fed a starter diet (1 to 28-day-old) with 3,300kcal ME/kg; 22.5% CP, and energy to protein ratio (EPR) of 146.7. The grower diet (29 to 42 days-old) had 3,300kcal ME/kg; 20.9% CP and EPR of 157.9, and the levels in the finisher diet (43 to 49-day-old) were 3,300kcal ME/kg; 18.2% CP and EPR of 181.3. Three restricted groups (R) were fed a low EPR diet having 2,800kcal ME/kg and 24% CP (EPR=117) from 7 to 14-day-old (R-1), 7 to 21-

day-old (R-2), or 21 to 35-day-old (R-3). All treatments were fed the control diet before and after the periods of feed restriction (Table 1).

Body weight gain, feed intake and feed conversion were weekly recorded. On the 14th,

21st, 35th and 49th day of age, liver samples were collected from one broiler per replicate. Histological sections were prepared, stained with Sudan III, and scored for liver fatty infiltration according to Lopez et al. (1982).

Table 1. Composition and chemical analysis of the experimental diets for broiler chickens

Ingredient (kg/ton)	Diet			
	Starter	Grower	Finisher	Restricted
Yellow Corn	599.93	719.74	787.81	586.04
Corn oil	65.77	45.17	34.34	-
Soybean meal	177.86	86.65	37.66	155.57
Fish meal	140.00	130.00	120.00	99.82
Wheat middlings	-	-	-	39.13
Sunflower meal	-	-	-	100.00
Sodium chloride	1.50	1.50	1.50	1.50
Oyster meal	11.94	12.25	11.91	11.58
Tricalcium phosphate	-	1.69	3.77	3.37
Supplement ¹	3.00	3.00	3.00	3.00
Calculated analysis (%) ²				
ME (kcal/kg)	3,300	3,300	3,300	2,800
Lysine	1.45	1.17	1.00	1.32
Methionine and cysteine	0.92	0.83	0.76	0.90
Tryptophan	0.29	0.23	0.20	0.30
Linoleic acid	4.81	3.86	3.37	1.34
Energy:protein ratio	146.7	157.9	181.3	116.2
Chemical analysis (%) ³				
Dry matter	90.3	87.5	89.3	90.0
Crude fiber	2.2	1.9	1.8	3.9
Ether extract	9.1	7.8	7.4	4.3
Nitrogen free extract	51.9	52.4	55.9	52.9
Ash	4.6	4.5	6.0	4.8
Crude protein	22.5	20.9	18.2	24.1

¹Provided per kg of diet: vit.A, 5,000IU; vit.D3, 2,000IU; vit.E, 11IU; vit.K, 0.75mg; vit.B1, 1.5mg; vit.B2, 4.5mg; d-pantothenic acid (100%), 12mg; niacin, 30mg; vit.B6, 2.5mg; choline chloride (50%), 110mg; folic acid, 1mg; biotin (2%), 4mg; vit.B12 (0.1%), 9µg; ethoxyquin (antioxidant), 125mg; olaquinox (100%), 20mg; Cu, 3.5mg; I, 0.17mg; Fe, 25mg; Zn, 30mg; Mn, 66mg; Se, 0.1mg; Co, 0.1mg. Composition informed by Feed Champion SA, Chile.

²Calculated values as suggested by González and Pokniak (1990).

³According to Methods... (1995).

Fatty infiltration was scored as light = (+: 1); little = (+: 2); mild = (+++: 3); and high = (++++: 4). Mean treatment scores were calculated as the sum of the products between the

number of broilers and the respective scores. Thus, mean scores ranged from 4 (four broilers with score 1) to 16 (four broilers with score 4). On the 49th day of age, the chemical composition

of the liver was evaluated following the AOAC procedures (Methods..., 1995).

Triglyceride (TGL) analysis was performed in blood samples collected from two broilers per replicate on the 14th, 21st, 28th, 35th, 42nd and 49th day of age. The birds had been fasted or not for 12 hours previously to blood sampling. The GPO-PAP enzyme methods¹ were used to measure TGL of the VLDL plus LDL fractions, as suggested by Segovia et al. (1990).

Data were submitted to analysis of variance (Model I) and mean differences were analyzed by Duncan's test (Sokal and Rohlf, 1981). Histology scores were evaluated using a nonparametric test (Fisher's exact test or Kruskal Wallis test).

Table 2. Performance of broilers fed control diet or submitted to qualitative restriction in different periods (mean ± SD)

Weeks of age	Treatment				P value*
	Control (C)	7-14d ¹ (R-1)	7-21d ¹ (R-2)	21-35d ¹ (R-3)	
Weekly weight gain (g)					
1	104±14.1	99±17.2	100±16.6	101±14.5	NS
2	226±33.8	218±20.3	212±31.2	226±29.3	
3	353±33.6a	338±43.6a	313±38.5b	347±37.2a	0.001
4	440±4.6a	439±61.3a	400±47.4b	348±59.9c	0.001
5	458±57.0a	446±59.4ab	416±79.2bc	400±67.1c	0.001
6	535±62.0ab	499±89.1bc	490±88.0c	544±65.1a	0.019
7	527±67.4	472±113.3	461±109.6	496±130.7	NS
Total	2653±205a	2521±260b	2395±275b	2477±241b	0.001
Weekly feed intake (g)					
1	106±6.1	102±7.9	103±4.7	101±5.2	NS
2	289±6.5	308±7.2	302±19.2	293±3.2	NS
3	496±13.4	499±7.0	520±30.0	497±4.1	NS
4	747±17.1	742±54.4	709±46.0	713±23.3	NS
5	934±27.7	904±71.1	855±29.3	937±73.5	NS
6	1196±17.0a	1109±62.2bc	1075±53.0c	1185±62.4ab	0.019
7	1282±43.3a	1147±96.9b	1121±104.0b	1200±32.2ab	0.051
Total	5049±60.5a	4813±249ab	4686±153.3b	4922±134.0ab	0.047
Weekly feed conversion					
1	1.02±0.03	1.03±0.01	1.02±0.01	1.00±0.05	NS
2	1.28±0.05a	1.42±0.02b	1.42±0.06b	1.30±0.02a	0.001
3	1.41±0.05a	1.48±0.05a	1.67±0.05b	1.44±0.09a	0.001
4	1.70±0.07a	1.69±0.05a	1.78±0.08a	2.06±0.12b	0.001
5	2.04±0.07a	2.02±0.021a	2.06±0.11a	2.38±0.26b	0.015
6	2.23±0.05	2.21±0.11	2.20±0.20	2.18±0.10	NS
7	2.43±0.04	2.46±0.27	2.45±0.10	2.43±0.10	NS
Total	1.90±0.01a	1.91±0.05a	1.96±0.03ab	2.00±0.06b	0.032

¹Period in which qualitative restriction diet was given [Energy to protein ratio (EPR=117)].

* Means followed by different letters in the same row are different (P<0.05). NS = non significant.

¹Merck test, Merck - Darmstet, Germany

RESULTS

Weekly weight gain (WWG) (Table 2) decreased in the groups subjected to qualitative restriction between 7 and 21-day-old (R-2), and between 21 and 35-day-old (R-3), when compared to control and R-1 groups. Feed restriction between 7 and 14-day-old (R-1) had no negative effects on WWG. Weight gain in R-2 was lower than in the control group after the restriction period and until five weeks (35-day-old), despite of a 15-day recovery. On the other hand, R-3 showed normal growth (P>0.05) as soon as the restriction period ended. On the other hand, total weight gain on the 49th day of age was higher (P<0.001) in the control group compared to restriction treatments. Differences (P>0.05) in weight gain among restricted groups at this age were not observed.

Qualitative restriction for 15d had different negative impacts on WWG according to the age of birds; impairment was greater due to restriction between 7 and 21-day-old animals (R-2). On the other hand, early restriction for one week (R-1) had no negative effects on weight gain.

Weekly feed intake (WFI) was not affected during the restriction periods ($P>0.05$). Conversely, feed intake was lower in R-1 and R-2 in comparison to the control group on 6th and 7th week of age ($P<0.019$ and $P<0.051$, respectively for 6th and 7th week of age). Total feed intake (1 to 49-day-old) in R-2 was lower than in the control group ($P<0.047$), but similar to the other restricted groups.

Weekly feed conversion (WFC) reflected weight gain and feed intake results, suggesting that feed conversion was impaired during the restriction period ($P<0.001$). However, this effect was not observed on the 6th and 7th week of age. Total

feed conversion was better in the control group and poorer in R-3 ($P<0.032$). There were no statistical differences at this age between the other treatments. Apparently, the evaluation period was not long enough for R-3 birds recover, and it is possible that rearing periods longer than 49 days would enable recovery in this group.

Liver histology was normal (Table 3), although intracellular fatty infiltration was observed. The liver showed visible and clean parenchyma, evident lobules, septum and sinusoids, besides clean and thin endothelial membranes (Fig. 1, 2, and 3). Hepatocytes were clear, with typical pyramidal structure, and normal polyploidy in some areas. The cells were normally involved by a microfibrillar net of reticular tissue. All features confirm a normal hepatic structure and suggest healthy functionality, in spite of the different fatty infiltration degrees that were observed.

Table 3. Scores of liver fatty infiltration (Sudan III stain) at the end of each qualitative restriction period and on the 49th day of age

Treatment	Score ¹			
	14d	21d	35d	49d
Control (C)	5 (3x1 + 1x2) ¹	4 (4x1)	5 (3x1+1x2)	14 (2x3+2x4)a
7-14d (R-1)	7 (3x1 + 1x4)			9 (1x1+1x2+2x3)ab
7-21d (R-2)		8 (4x2)		8 (1x1+2x2+1x3)ab
21-35d (R-3)			12 (4x3)	6 (2x1+2x2)ab
$P\leq^*$	1.00 ²	0.029 ²	0.029 ²	0.018 ³

*Means followed by different letters in the same columns are different ($P<0.05$).

¹Score means (sum of the products between the number of affected birds and score: see Material and Methods).

²Fisher's Exact Test.

³Kruskal Wallis Test.

At the end of the different restriction periods (Table 3), higher degrees of fatty infiltration were observed in the hepatocytes of restricted birds in relation to control birds. According to histology scores, intracellular fat was 40%, higher for R-1, 100% for R-2 and 140% for R-3 compared to control birds. However, this tendency was reverted on the 49th day of age. Intracellular fatty infiltration scores decreased to

35.7%, 42.9%, and 57.1% for R-1, R-2, and R-3, respectively.

Proximate analysis of the liver samples collected on the 49th day of age (Table 4) is in accordance with the scores of intracellular fatty infiltration shown in Table 3.



Figure 1. Photomicrograph of broiler chicken liver tissue stained with Sudan III showing light (+) fatty infiltration (100x magnification).

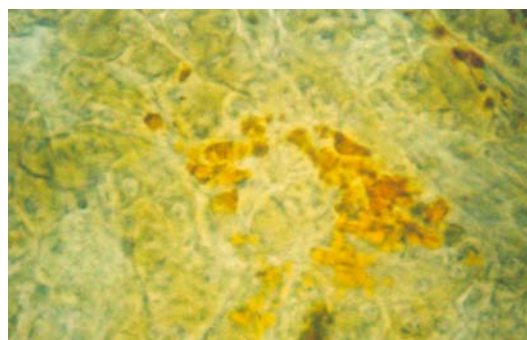


Figure 2. Photomicrograph of broiler chicken liver tissue stained with Sudan III showing little (++) fatty infiltration (400x magnification).

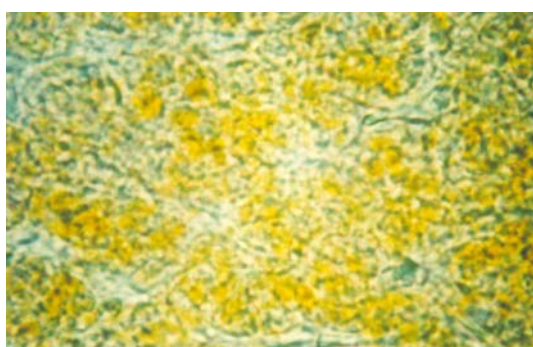


Figure 3. Photomicrograph of broiler chicken liver tissue stained with Sudan III showing mild (+++) fatty infiltration (100x magnification).

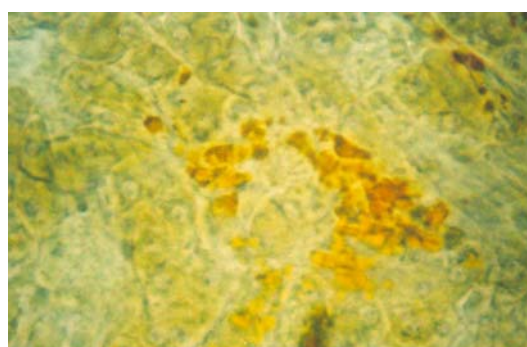


Figure 2. Photomicrograph of broiler chicken liver tissue stained with Sudan III showing little (++) fatty infiltration (400x magnification).

Table 4. Proximate analysis of the liver (fresh matter basis, %) at 49 days of age after different periods of qualitative restriction (mean±SE)

Treatment	Dry matter (DM)	Crude protein (CP)	Ether extract (EE)
Control	34.8±3.2	19.4b±1.6	10.3a±3.0
7-14d (R-1)	32.1±3.1	20.6ab±1.6	6.6b±2.3
7-21d (R-2)	31.9±2.3	22.9a±2.00	5.5b±2.0
21-35d (R-3)	29.7±1.3	20.3ab±1.4	4.8b±1.3
p≤*	NS	0.056	0.020

*Means followed by different letters in the same column are different (P<0.05).

Lower ether extract content (P<0.02) was observed in the liver of restricted birds at the end of the trial in comparison to control birds. Fat levels were also lower in the treatments with shorter periods to recover from restriction. Crude protein levels tended to increase in the restricted groups, and the difference between R-2 and control was almost significant (P>0.56).

Triglyceride levels were not different (P>0.05) between treatments (Table 5), except for non-fasted birds from R-2 compared to control birds

(P<0.05) on the 28th day of age. As expected, broilers subjected to 12 hours of fasting showed triglyceride levels lower than non-fasted chicks. It is worth mentioning that the lower TGL values obtained in 21-day-old broilers were quite similar to those obtained for the fasted broilers.

DISCUSSION

Qualitative restriction decreased weight gain during the rearing period. Compensatory growth was not stimulated by such decrease, as observed

by the lower total weight gain of restricted birds compared to control birds (1 to 49-day-old, Table 2). Conversely, Plavnik and Hurwitz (1991) reported compensatory growth in birds submitted to more stringent conditions of restriction. Body weight reduction was reported to be 26% and 36%, i.e., greater than the values observed in the

present study. Apparently, greater reductions in weight gain are needed to trigger the physiological mechanisms of compensatory growth (Boza et al., 1999). On the other hand, Zubair and Leeson (1996) subjected broilers to severe early feed restriction and did not observe compensatory growth on the 49th day of age.

Table 5. Plasma triglycerides (mg/dl) in broilers subjected or not to fasting for 12 hours

	Age (d)					
	14	21	28	35	42	49
Fasted						
C	20.0±3.2	14.0±1.8	28.3±2.5	22.5±9.1	11.3±2.1	19.0±7.4
R-1	18.0±3.5	18.5±5.6				15.3±5.0
R-2		20.3±8.3	24.5±4.2			14.0±5.0
R-3				19.3±6.9	14.3±4.0	12.0±1.2
Non-fasted						
C	100.3±25.6	27.0±7.5	128.0±20.2a	63.5±14.7	118.5±8.1	66.8±32.4
R-1	73.8±24.5	18.5±6.0				42.3±7.4
R-2		21.8±2.2	71.0±38.2b			73.5±28.8
R-3				44.5±18.0	90.5±31.0	63.5±7.5

*Means followed by different letters in the same column are different (P<0.05).

Restricted broilers have not increased feed intake to compensate for the lower dietary energy levels. Therefore, energy and protein intake was lower in restricted birds compared to control birds and resulted in lower weight gain. These results corroborate the findings of Bartov and Plavnik (1998) who reported that intake in broilers is very close to the maximum capacity of the digestive tract. However, Leeson et al. (1992) observed an increase in feed intake during qualitative feed restriction in older broilers.

The lower feed intake observed in treatment R-2 at the end of the experimental period (Table 2) might have been associated to the smaller development of the digestive tract as a result of decreased final body weight (49-day-old).

The feed conversion of restricted groups was poorer immediately after the restriction periods. Besides, there was a residual effect on feed conversion at the end of the trial (49-day-old), so that poorer feed efficiency was observed in R-2 and R-3. This was expected, since weight gain was lower and there were no differences in feed intake compared to control birds. Boza et al. (1999) have suggested that poorer efficiency might result from the absence of compensatory

growth, similar to what was observed in the present study.

The greater intracellular fatty infiltration in the liver at the end of each restriction period (Fig. 1, 2, 3, 4, and Table 3) might suggest that mechanisms of "de novo" synthesis of endogenous fat had been triggered in the liver. Tanaka et al. (1983b) reported that higher levels of dietary carbohydrates induced more lipid synthesis in the liver, which might explain the results of greater fatty infiltration. In the present study, control chicks were fed a 10.1 to 8.3 % of ether extract (100% DM) diet, and the restriction diet contained only 4.8% of ether extract (Table 1). Therefore, since there were no differences in feed intake, fat intake in restricted groups was 52% lower than in the control group. Moreover, crude protein levels in the control diets (100% DM) varied from 24.9 to 23.9%, whereas CP level was 26.8% in the restriction diet. As feed intake was similar between the groups, mean protein intake was 7.6 to 11.2% higher during the restriction periods. On the other hand, there were minor differences between treatments (7% in average) in the levels of easily digestible carbohydrates (non-nitrogenous extract, NNE)

compared to the differences in protein between control and restricted birds. Therefore, considering the chemical composition of the diets and what has been reported by Tanaka et al. (1983a,b), it might be assumed that both the lower fat intake and the higher CP and NNE in the restricted diets increased fat synthesis in the liver, and might have affected hepatic fatty infiltration at the end of each restriction period. Nevertheless, it was observed that earlier restriction induced less fatty infiltration on the 49th day of age, which was confirmed by the proximate analysis of the liver. On the contrary, it is possible that the differences between restricted and control groups on the 49th day of age were simply a result of the higher level of fat deposition in the control treatments.

Since there were no statistical differences in TGL levels between treatments at the evaluated ages (Table 5), the changes in hepatic lipid synthesis are not evident. On the 49th day of age, TGL concentration was slightly lower in plasma collected from previously restricted birds. As expected, values in fasted birds were lower and standard deviation was smaller than in non-fasted birds. These results are in accordance to Griffin et al. (1982), who reported that diet effects should be avoided in studies where TGL levels are to be determined. On the 49th day of age, triglyceride levels in the plasma of non-fasted control birds (66.8±32.4ml/dl) were similar to the level of 65.6±26ml/dl reported by Whitehead and Griffin (1982).

Finally, the histological procedures used herein allow clear differentiation of the degrees of hepatic fatty infiltration as a result of qualitative restriction. The results showed very good correlation with hepatic chemical composition, although there was poor correlation with metabolic events (TGL results).

In conclusion, the qualitative restriction resulted in weight gain reduction between 12.1% and 16.7%. The reduction in weight gain was not enough to stimulate compensatory growth. Qualitative restriction affected both fatty infiltration and proximate analysis of the liver. A new and straightforward method should be developed in order to enable better understanding of the metabolic events concerning fat biosynthesis associated with dietary management in broilers.

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