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ALTERAÇÃO DA MASSA CORPORAL E DO CONSUMO ALIMENTAR DE RATOS SUPLEMENTADOS COM WHEY PROTEINS

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RESUMO

Introdução: As whey proteins tem recebido grande atenção por conta de seus benefícios sobre a hipertrofia muscular esquelética, redução de gordura corporal, performance, regulação do apetite, regulação do sistema imunológico, regulação do perfil lipídico e combate ao estresse metabólico. Diante disso, a sua importância muitas vezes é superestimada, levando atletas e indivíduos praticantes de atividades físicas a consumirem doses muito elevadas de proteínas, sem a garantia de benefícios adicionais. Objetivo: Quantificar durante 12 semanas a alteração do consumo de ração e da massa corporal total de ratos wistar suplementados por whey proteins nas doses de 2, 4, 6g/kg/dia. Materiais e Métodos: Protocolo de aprovação da Comissão de Ética no Uso de Animais da Universidade Federal do Maranhão nº 23115.014424/2015-54). Foi adotado delineamento de pesquisa experimental com os ratos sendo distribuídos aleatoriamente em 4 grupos: Grupo Controle (C), Grupos suplementados (W2, W4, W6); o estudo foi realizado com 38 Rattus novergicus machos da linhagem Wistar com idade inicial de 60 dias e massa corporal entre 218 e 323 gramas; a suplementação com whey proteins dissolvidas em água seguiu as dosagens 2, 4 e 6 g/kg/dia a suplementação foi realizada durante 12 semanas, diariamente, três vezes por dia, em volume de 5ml, com uma hora de intervalo entre cada gavagem. As variáveis foram testadas quanto à distribuição utilizando o teste de Shapiro-Wilk (p>0,05). Os dados do consumo de ração, foram analisados pelo teste ANOVA; a variável de classificação foi a interação entre o consumo de ração ou massa corporal e a suplementação (C, W2, W4, W6); o pós-teste de Tukey foi utilizado para determinar as diferenças estatísticas entre os grupos (C, W2, W4, W6), utilizou-se o software GraphPad Prism 7. Resultados: A média de consumo de ração no grupo controle não apresentou diferença estatística significativa em relação ao grupo Whey 4 nas semanas 1 (p=0,3450), 2 (p=0,1027), 3 (p=0,1595), 4 (p=0,5572) e 5 (p=0,2539). Houve diferença estatística significativa no aumento da massa corporal total do grupo controle em comparação ao grupo Whey 4 nas semanas 10 (p=0,0454), 12 (p=0,0348) e na semana final (p=0,0138). Do mesmo modo, houve diferença estatística significativa no aumento da massa corporal total do grupo controle em comparação ao grupo Whey 6 na semana final (p=0,0048). Nesse mesmo sentido, houve diferença estatística significativa no aumento massa corporal total do grupo Whey 2 em comparação ao grupo Whey 6 nas semanas 1 (p=0,0210), 2 (p=0,0434) e na semana final (p=0,0289). Discussão: Dietas com altos teores de proteínas desempenham um papel importante no surgimento da saciedade em função do seu envolvimento com a produção a nível intestinal de diversos hormônios anorexígenos que por sua vez estimulam o nervo vago, promovendo estímulos neuronais para o núcleo trato solitário, sinalizando saciedade. Em função disso, a ingestão a longo prazo de uma dieta rica em proteínas diminui não só a ingestão de alimentos, mas também a massa corporal e a adiposidade corporal em ratos. Conclusão: A suplementação com as proteínas do soro do leite nas doses de 4 e 6g/kg/dia em ratos sedentários conferiram estabilidade em relação a massa corporal total e redução significativa do consumo de ração ao longo de 12 semanas. A suplementação com as proteínas do soro do leite na dose de 2g/kg/dia em ratos sedentários não promoveu redução no consumo médio de ração e apresentou efeito semelhante as demais doses sobre a massa corporal total.

Palavras-chave: Whey Proteins. Consumo de Ração. Massa Corporal. Saciedade.

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ABSTRACT

Alteration of food consumption and body mass of whey proteins supplemented rats

Introduction: Whey proteins have received great attention because of their benefits on skeletal muscle hypertrophy, body fat reduction, performance, appetite regulation, immune system regulation, regulation of the lipid profile and the combat against metabolic stress. In this sense, their importance is often overestimated, leading athletes and physical activity individuals to consume very high doses of protein, without the guarantee of additional benefits. Aim: For 12 weeks quantify the evolution of food intake and total body mass of Wistar rats supplemented with whey proteins with dosages of 2, 4, 6g/kg/day. Materials and methods: Approval protocol of the Ethics Committee on the Use of Animals of the Federal University of Maranhão nº 23115.014424 / 2015-54). An experimental research design was adopted with the rats being randomly distributed in 4 groups: Control Group (C), Supplemented groups (W2, W4, W6); the study was performed with 38 male Rattus novergicus Wistar rats with initial age of 60 days and body mass between 218 and 323 grams; supplementation with whey proteins dissolved in water followed the dosages 2, 4 and 6 g / kg / day supplementation was performed for 12 weeks, daily, three times a day, by volume of 5 ml, with an hour interval between each gavage. Variables were tested for distribution using the Shapiro-Wilk test (P> 0.05). The data of the feed consumption were analyzed by the ANOVA test; the classification variable was the interaction between feed consumption or body mass and supplementation (C, W2, W4, W6); the Tukey post-test was used to determine the statistical differences between the groups (C, W2, W4, W6), GraphPad Prism 7 software was used. Results: The mean intake of rations in the control group did not present a statistically significant difference in relation to the Whey 4 group at weeks 1 (p=0,3450), 2 (p=0,1027), 3 (p=0,1595), 4 (p=0,5572) and 5 (p=0,2539). There was a statistically significant difference in the increase of the total body mass of the control group compared to the Whey 4 group at weeks 10 (p=0,0454), 12 (p=0,0348) and at the final week (p=0,0138). Likewise, there was a significant statistical difference in the increase of the total body mass of the control group compared to the Whey group 6 in the final week (p=0,0048). In the same sense, there was a statistically significant difference in the total body mass increase of the Whey 2 group compared to the Whey 6 group in weeks 1 (p=0,0210), 2 (p=0,0434) and in the final week (p=0,0289). Discussion: Diets with high protein content play an important role in satiety due to its involvement with intestinal production of several anorectic hormones, which in turn stimulate the vagus nerve, promoting neuronal stimuli to the solitary tract nucleus, signaling satiety. As a result, the long-term ingestion of a high-protein diet reduces not only food intake, but also body mass and body adiposity in rats. Conclusion: Supplementation with serum whey proteins at doses of 4 and 6 g/kg/day in sedentary animals conferred stability over total body weight and a significant reduction in feed intake over 12 weeks. Supplementation with serum whey proteins at 2 g/kg/day in sedentary animals did not lead to a reduction in the average feed intake and showed a similar effect to the other doses on total body weight.

Key words: Whey Proteins. Feed Intake. Body Mass. Satiety.

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INTRODUCTION

About the Consumption of Whey Proteins

According to World Health Organization (2003), the recommendation for total protein consumptions ranges from 10 to 15% of the total energy value. In this sense, according to Dietary Recommended Intake, the protein consumption for adult men and women ranges to 0.8 to 1.0g/kg/day of high biologic value protein. Then, it is considered a high protein diet, when protein consumptions occur around 1,8 to 3,3g/kg/day (Lancha Junior, Campos-Ferraz e Rogeri, 2009; Institute of Medicine, 2006).

Therefore, the consumption of complete proteins, especially as sources of amino acids is indispensable to adequate function of many metabolic process, among which is highlighted the food consumption, that is sensitive in relation to protein content of the diets and it is regulated in several peripheral stages, such as the secretion of intestinal neuropeptides, insulin and metabolites derived from amino acids that converge to the central nervous system, suggesting that an increase in the protein content of the diet can reduce the energy intake (Journel et al., 2012; Bensaid et al., 2003; Feurte et al., 1999).

Thereat, the Australian Commission of Sport (2009), in their guidelines, recommend changes above the reference values in the dietary protein content in athletes wishing to maintain the shape, to change the body composition and those who will undergo for a reduction in training volume, since the increase of the dietary protein content promotes the decrease of the appetite, hunger, in addition, it helps in the reduction in the punctual consumption and of the total energy content of the diet.

Among the dietary protein, milk proteins are considered high quality proteins and are wisely studied because of its many benefits, these proteins comprise two major portions, casein, that represent 80% of the protein content and whey protein, that represent 20% of the content (Pichon et al., 2008).

Thereat, whey proteins, in especial, have received great attention because its benefits on skeletal muscle hypertrophy, reduction in body fat, performance, appetite regulation, immune system regulation, lipid profile regulation and metabolic stress decrease (Krissansen, 2007).

Probably, because of that, the national industry of nutritional supplements has reached a growth of 10% in its production in 2016, with a billing around 1.49 billion Brazilian Real (Brasnutri, 2016).

In relation to protein supplements, it is understood that proteins are vital elements to a successfully promotion in the process of muscle hypertrophy and in the reduction of body fat, especially due the exercise with overload. Being this importance, many times overestimated, leading athletes, and practicing individuals of physical activities to consume very high doses of proteins, can reach values ranging from 4.0 to 6.2g/kg/day (Bacurau, 2009).

The present study is necessary to investigate the behavior on the change in body mass and in the food consumption of supraphysiologic doses of whey proteins, to identify if doses greater than those recommended provide additional benefits on the body mass and food consumption.

It was hypothesized that whey proteins supplementation in doses of 2, 4 and 6g/kg/day doesn't promote an increase in the feed consumption and total body weight over 12 weeks in wistar rats.

Therefore, the aim of this experiment was to quantify, for 12 weeks, the change in the feed consumption and in the total body mass of wistar rats supplemented with whey proteins in doses of 2, 4 and 6g/kg/day.

MATERIALS AND METHODS

Ethical Considerations

The biological assay was based in the Use and Care Guide to Laboratory Animals of the Brazilian College in Animal Experimentation and the experimental protocol received initial substantiated opinion with approval and protocol n° 23115.014424/2015-54 in the Ethic Commission on Animal Use of the Federal University of Maranhão - UFMA.

Study Design

For the present study was adopted a experimental research design with randomized groups, divided according to the received supplementation: (C) Control Not-supplemented; (W2) Supplemented with 2g/kg/day of whey proteins; (W4) Supplemented with 4g/kg/day of whey proteins;

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and (W6) Supplemented with 6g/kg/day of whey proteins.

Study place

The experimental procedure happened in 12 weeks, inside the Sectorial Bioterium of the building of Post-Graduate in Biological Science and Health, located in Dom Delgado University Town, Bacanga Campus, Federal University of Maranhão - UFMA.

Samples

The present study was performed with 38 males Rattus novergicus albinus Wistar rats with and initial age of 60 days and an initial body mass between 218 and 323 grams, originated from the Vivarium of the Federal University of Maranhão - UFMA.

The rats stood on hygienic conditions in metabolic cages, kept in a climatized laboratory with temperature control between 24 to 28 °C, under light/dark cycle of 12 hours. They were fed with balanced ration standard to rodents (Labina®) and ad libitum water.

The rats from each group were differenced by tracer tagging in the tail with gentian violet.

Procedures

The supplementation with whey proteins was performed by gavage during 12 weeks, daily, into three times a day in volume of 5 ml, 1 hour-interval between each gavage. The Control group received filtered water at the same frequency and volume as the intervention group.

The control of feed consumption occurred daily, being made available at the end of the experimental protocol 300 grams of feed for free consumption and, after 24 hours, before the beginning of the experimental protocol a residual feed weighing was made.

Weekly, the body mass of the rats was evaluated for readjustment of the whey proteins supplementation dose.

Statistic

The variables described were tested for normal distribution using the Shapiro-Wilk test (P> 0.05). The quantitative data of the feed consumption were analyzed by the two-way ANOVA; the classification variable was the interaction between diet and supplementation (C, W2, W4, W6).

The Tukey's post-test was used to determine the statistical differences between groups (W, W2, W4, W6). The software used for the analyzes was GraphPad Prism 7.

RESULTS

As observed in the figure 1, the mean of feed consumption in the Control group did not show a statistically significant difference in relation to the Whey 4 in the weeks 1 ($p=0.3450$), 2 ($p=0.1595$), 4 ($p=0.5572$) and 5 ($p=0.2539$). About the others weeks, there was a significative statistic difference ($p<0.05$) in the feed consumption between the control, Whey 2, Whey 4 and Whey 6 groups.

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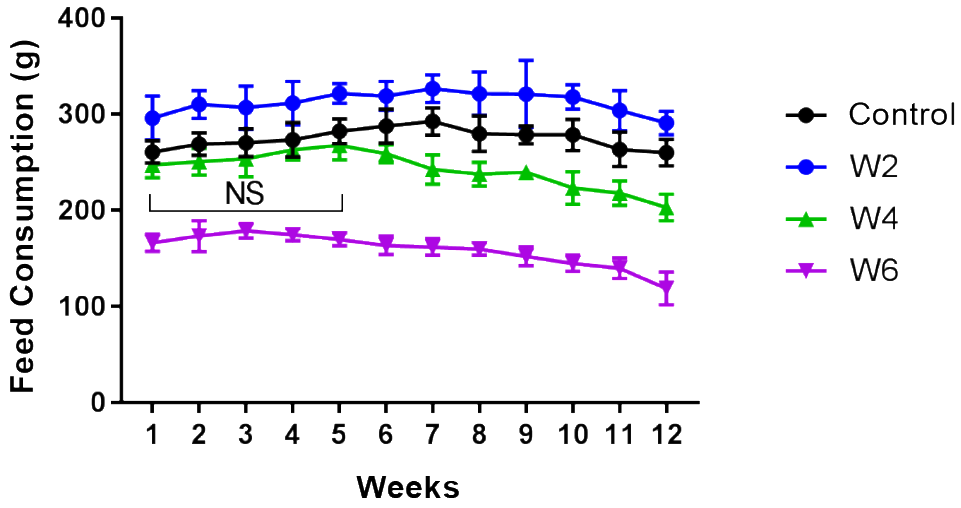

Figure 1 - Feed Consumption per week of the groups.

Thereat, there was a decrease in the mean feed consumption of the groups as the supplemented dose of whey protein were bigger, suggesting that a higher protein consumption is associated to a lower food intake, possibly because of the involvement of satieties mechanisms stimulated by protein consumption.

However, the Whey 2 group presented a mean feed consumption statistically higher ($p<0.05$) than Control group what suggest that the supplementation of 2g/kg/day of whey proteins was not able to reduce the mean feed consumption in rats.

As observed in the figure 2, there was statistically significant difference in the increase in total body mass of the control group compared to Whey 4 group in the weeks 10 ($p=0.0454$), 12 ($p=0.0348$) and in the final week ($p=0.0138$). Likewise, there was a statistically significant difference in the total body mass increase of the Control group compared to Whey 6 group in the final week ($p=0.0048$).

In the same way, there was a statistically significant difference in the total body mass increase of the Whey 2 group compared to Whey 6 group in the weeks 1 ($p=0.0210$), 2 ($p=0.0434$) and in the final week ($p=0.0289$).

In other points analyzed, there was not a statistically significant difference ($p>0.05$) between the Control, Whey 2, Whey 4 and Whey 6 groups in all the weeks.

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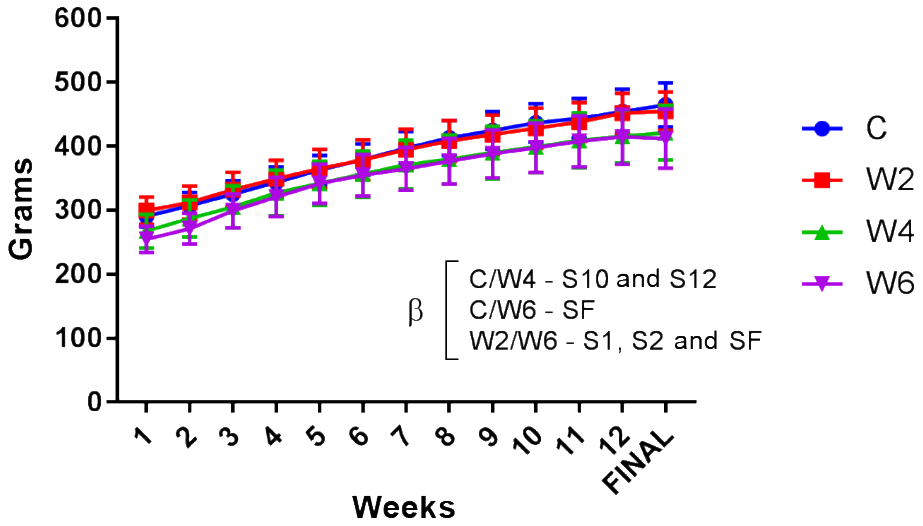

Figure 2 - Body mass per week of the groups.

Therefore, it was observed that the behavior of total body weight gain of the groups presented similarly, however, especially in the W4 and W6 groups, a stabilization in total body mass gain was observed over time, suggesting that the higher consensus is related to lower food consumption, which results in the stabilization of total body mass.

In addition, the W2 group showed similar behavior to the control, suggesting that the supplementation of 2g / kg / day of whey proteins was not able to change the total body mass of the rats.

DISCUSSION

Journel et al., (2012), in a systemic review study about the brain repercussions as a function of high-protein diets, concluded that proteins perform an important role in the emergence of satiety and that the intake of a long term of a diet rich in protein reduces not only the diet intake, but also body mass and body adiposity in rats and in human being. In addition, they infer that after consumption of a high-protein diet, the intestine is an anorectic hormone, among them the cholecystokinin, which stimulates the vagus nerve, promoting neuronal stimulus to the solitary tract nucleus, signaling satiety.

The inferences reported by Journel et al., (2012), were not similar to the results of the present study since there was no behavior to reduce body mass or to reduce food intake.

However, the pathways involved in the physiological response to high protein consumption are the same, explaining the stabilization behavior in feed consumption, whey protein consumption and body mass gain reported in the present study.

In parallel, Pichon et al., (2008), in an experimentation with a duration of 25 days, containing 10 groups with 8 male wistar rats each, with body mass between 290 and 295 grams, aged 12 weeks, organized in a control group, which received a diet P14C76L10 (a standard diet consisting of 14% proteins, 76% carbohydrates and 10% lipids) P55C35L10 group (high protein diet consisting of 55% proteins, 35% carbohydrates and 10% lipids) P55C15L30 group (diet consisting of 55% proteins, 15% carbohydrates and 30% lipids) and P55L45 group (diet composed of 55% of proteins and 45% of lipids).

Due to this, within each model of high protein diet adopted, there are 3 formulations that correspond to the following proteins: milk protein (composed of 85% casein and 15% whey proteins), whey protein concentrated, and protein isolate from milk rich in native β-lactoglobulin. The control group consumed the standard feed throughout the study period, while the high protein diet groups consumed the standard diet during the first 10 days, and then consumed their respective diets until the end of the experimental protocol.

In function of this, it was reported that, in relation to feed consumption, when

analyzing, in insolation, the P55C35L10 diet, there was a statistically different significance in the consumption reduction in whey proteins groups ($p<0.01$) and β-lactoglobulin ($p<0.01$) when compared to milk protein group.

Likewise, when analyzing the P55L45 diet, there was a statistical difference in the consumption reduction in whey protein groups ($p <0.01$) and β-lactoglobulin ($p <0.01$) compared to the milk protein group. However, when analyzing the P55C15L30 diet, no statistical difference was reported between the groups, whereas in the control group, feed consumption was statistically higher ($p <0.01$) when compared to the high protein diet groups.

Regarding to body mass, the control group presented a statistically superior gain ($p <0.01$) of body mass in relation to the high protein diet groups. In this sense, the body mass gain was statistically lower ($p<0.01$) in the β-lactoglobulin protein groups, regardless of the carbohydrate / lipid ratio of the diet. In addition, when analyzing the P55L45 diet, it was presented statistically lower weight gain ($p<0.01$) in the whey proteins ($p <0.01$) and β-lactoglobulin ($p<0.01$) groups compared to group of milk protein, which presented only one trend.

Additionally, if each combination is analyzed in isolation, it can be seen that the β-lactoglobulin protein group in the P55L45 diet obtained a statically lower weight gain ($p<0.01$) in relation to the other groups and that, as regards to feed consumption, it can be seen that the highest statistical consumption reduction was observed in the whey proteins ($p<0.01$) and β-lactoglobulin groups ($p<0.01$) in the P55L45 diet.

The founds of Pichon et al., (2008), suggest that the high consumption of dietary protein was able to interfere on feed consumption and consequently on muscle mass gain, moreover, they open a perspective to the actions of different types of proteins, associated or not, to carbohydrates and fats, on satiety and food behavior, the possible effect of these many dietary models.

These found are similar to this present study in sense that the high protein content was able to interfere on feed consumption and body mass stabilization suggesting that the action of satietogen effect promoted by protein, however, Pichon et al., (2008), used a same dose of protein for every group, while the present study used distinct doses and crescent in order to evaluate the response to the consumption of supraphysiologic doses of whey proteins.

Bensaid et al., (2003), in an experiment with 24 days of duration, consisting of 16 males wistar rats, with body mass between 200 and 220 grams, organized into intervention group, received the feed P14 (consisting of 140g/kg of milk protein) during 10 days and after during 14 days they received the feed P50 (consisting of 500g/kg of milk protein), and the control group received the standard feed (consisting of 140g/kg of protein), they reported that, the introduction of higher protein content induced to a immediate and important reduction in feed intake, with later adaptation with the advance of the protocol.

In the first day, there was a significative reduction of 48% in total food consumption ($p<0.01$), in the second day it was of 28% ($p<0.01$) and in the fourteenth day it was of 18% ($p<0.05$) in comparation to the last day of the P14 diet.

Moreover, the number of meals was significative lower ($p<0.05$) after the adaptation to the P50 diet, suggesting that the rich protein diet did not induce aversion to the feed consumption of the rats, only granted better satiety.

The founds of Bensaid et al., (2003), suggest that the consumption of a high protein content diet is able to change in a short period of time the food behavior in relation to the feed consumption, suggesting that the actioned mechanism was of satiety not food aversion, which can occur in function of low palatability of protein preparation. This mechanism of satiety promotes the body mass regulation, since that induce to reduce the total energy intake.

Even supplemented doses and time of protocol used in this present study being superior to Bensaid et al., (2003), the founds are similar to the present study, because the consumption of high protein diet proved be effective in feed consumption reduction and for consequence on gain stabilization of the total body mass.

CONCLUSION

Whey protein supplementation in doses of 4 and 6g/kg/day in sedentary rats conferred stability in relation to the total body mass and significative reduction of the feed consumption over 12 weeks.

Whey proteins supplementation in doses of 2g/kg/day in sedentary rats did not promote reduction in the mean feed

consumption and presented similar effect the others doses on body total mass

CONFLICT OF INTERESS STATEMENT

The authors declare that there is no conflict of interest on any aspect.

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Recebido para publicação em 29/07/2022
Aceito em 03/09/2022