

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com



Research Article

Economic Analysis and Egg Yolk Cholesterol of Local Ducks Fed a *Salvinia molesta* and *Brotia costula* Combination

Siswanto Imam Santoso, Bambang Mulyatno, Sudiyono Marzuki, Edjeng Suprijatna and Agus Setiadi

Faculty of Animal and Agricultural Sciences, Diponegoro University, 50275 Semarang, Central Java, Indonesia

Abstract

Background and Objective: Eggs are common foods that are delicious and nutritious, but it had negative aspect of duck eggs that is their cholesterol content. One approach to improving the fat composition of eggs is feed supplementation. The study aimed to evaluate the effects of *Salvinia-Brotia* (SB) (at various percentages) as a duck feed supplement on the lipid composition (total cholesterol, LDL, HDL and triglyceride levels) of duck eggs. **Materials and Methods:** The study was carried out according to the guidelines for applied nutrition experiments in poultry. Starter periods of 120 birds 5 week-old Pengging ducks with an average body weight of 734.25 ± 0.52 g were maintained until they were 12 weeks old. The animals were housed in groups under standard conditions in a 20-unit postal cage with 4 individuals/unit. The ducks were fed 3 times/day ad libitum with 2,800 kcal of a metabolic energy and 18% crude protein. Their fattening duration was 49 days. Individuals were chosen for the experiment by a completely randomized design with 4 treatment groups and 6 replicates: T₀ (basal diet), T₁ (10% SB), T₂ (12.5% SB) and T₃ (15% SB). **Results:** SB supplementation in feed for 8 weeks did not significantly affect the body weight or physical characteristics of duck eggs. The addition of 12.5% of SB (T₂) lowered the cholesterol compared to the 10% treatment (T₁), while this did not differ significantly with the addition of as much as 15% SB (T₃). Furthermore, SB supplementation also significantly lowered the LDL content in egg yolks and the provision of 12.5% SB (T₂) resulted in the lowest LDL and triglyceride content among the treatments, accompanied by increasing contents of HDL. Utilization of the weed *Salvinia molesta* and the weed's waste water *Brotia costula* as a feed supplement can effectively improve the quality of local duck eggs. **Conclusion:** The incorporation of 12.5% *Salvinia-Brotia* (SB) gave the best results in the duck diets would reduce the total cholesterol, feed cost and finally could increase the income over feed cost.

Key words: *Salvinia molesta*, *Brotia costula*, egg yolk, cholesterol, economic analysis

Received: June 15, 2017

Accepted: August 01, 2017

Published: August 15, 2017

Citation: Siswanto Imam Santoso, Bambang Mulyatno, Sudiyono Marzuki, Edjeng Suprijatna and Agus Setiadi, 2017. Economic analysis and egg yolk cholesterol of local ducks fed a *Salvinia molesta* and *Brotia costula* combination. Pak. J. Nutr., 16: 684-689.

Corresponding Author: Agus Setiadi, Faculty of Animal and Agricultural Sciences, Diponegoro University, 50275 Semarang, Central Java, Indonesia

Copyright: © 2017 Siswanto Imam Santoso *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Duck production plays an important role in the agribusiness field in many Asian countries¹. Indonesia currently possesses a population of more than forty-four million ducks, as duck meat is popular and is in strong demand in throughout society². One of the indigenous Indonesian ducks that is commonly cultivated by farmers is the Pengging duck (*Anas javanica*). It is a dual purpose type of poultry that provides high yields of meat in addition to eggs¹. People who pay attention to healthy lifestyles desire duck eggs of good quality.

The duck eggs are common foods that are delicious and nutritious. Eggs are among the most nutritious foods in the world and are also the most recommended. Several types of eggs are the most widely consumed, namely, chicken eggs, leghorn chicken eggs, quail eggs, turkey eggs and duck eggs. Duck eggs are actually far superior to chicken eggs, with the same flavor but richer in nutrients. This is probably also influenced by the ducks' food, where their maintenance with free feeding choice in the field certainly allows them to acquire organic and natural foods.

The negative aspect of duck eggs is relatively higher cholesterol content, which is 2-fold higher than chicken egg. That is why people with a history of heart disease should be careful about consuming duck eggs. Healthy people should restrict themselves to simply one egg a day. This may differ from person to person, because the energy and caloric needs are determined by the activity of each individual. The cholesterol in duck eggs is needed much more by athletic individuals or workers who rely on physical labor. Meanwhile, those who are inactive or obese need to limit the high calories of duck eggs.

One approach to improve the fat composition of eggs is feed supplementation. However, the problems associated with feed often become obstacles to duck cultivation in the local poultry industry. Furthermore, in an intensive farm, feed costs can reach 60-70% of the total production cost^{1,3,4}. Therefore, a cheap alternative feedstuff that does not compete with the resources for human needs is required. In addition, the material also must be abundant such that its existence and production continuity are maintained. This study is the first research that combines *Salvinia* and *Brotia* in the rations.

Based on these requirements, one species of water fern, namely, Invasive Giant Salvinia, *Salvinia molesta* (SM), is a suitable alternative feed. A previous study demonstrated that SM is a good source of minerals and essential amino acids in feedstuff for ducks⁵. Some studies utilizing the water plant have been performed^{4,5}. However, its digestible energy and

protein content are low because the crude fiber content of SM is high. Consequently, such features can restrict pig production. It requires fermentation using *Aspergillus niger* (AN) to decrease the crude fiber content and optimize the applicability of SM as a feedstuff⁶.

One species of seaweed, *Gracilaria* sp., has waste that is rich in carbohydrate⁷. In addition, species of the genus *Brotia*, Southeast Asian freshwater snails that live as *Gracilaria*'s symbionts, are often considered as seaweed wastes that are rich in calcium and protein⁷. Therefore, *Gracilaria*'s wastes (GW) apparently have potential as a feed supplement that have an effect on either the performance or the meat quality of farm animals. However, it has not been reported that GW can be used as a feed supplement. During this time, by using a fermentation process, SW has only been used as an organic fertilizer and a source of the bacterial genus *Gracilibacillus* to produce alginate lyase degradation enzyme for degrading alginate to oligosaccharides in wakame during the composting process⁸. Moreover, as a floating residue, SW shows excellent prospects as a potential feedstock for the production of bioethanol through the fermentation process by using *Saccharomyces cerevisiae*⁹. Thus, investigations to examine the potential of GW as an efficient feed supplement are necessary to achieve the desired performance and product quality in farm animals.

Thus, the present study was designed to investigate the effect of a *Salvinia molesta* and *Brotia costula* combination as a feedstuff on the meat characteristics in ducks for the first time. Therefore, the study aimed to evaluate the effects of SB (at various percentages) as a duck feed supplement on the lipid composition (total cholesterol, LDL, HDL and triglyceride levels) of duck eggs. This study is an attempt for better utilizing invasive or waste-plants indirectly to overcome the health problems associated with certain egg products.

MATERIALS AND METHODS

Preparation of diet: The study was carried out at the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia, according to the guidelines for applied nutrition experiments in poultry¹⁰. Starter and finisher periods of 4 week-old, 80 Pengging ducks with an average body weight of 734.25 ± 0.52 g were chosen for this study. They were fed SB (Table 1). *Salvinia molesta* (SM) was collected from Rawa Pening Lake, Central Java, Indonesia. *Brotia costula* (BC) was collected from Brebes, Central Java. After removal of its roots, SM was dried under sunlight and then powdered. Fermentation was performed aerobically by using *Aspergillus niger* (AN) with an SM:AN ratio of 1000:12 g mixed

Table 1: Ingredients and composition of the experimental diets on the starter period

Feed stuff	<i>Salvinia-brotia</i> (% of diet)			
	T ₀	T ₁	T ₂	T ₃
Corn	53.60	48.10	47.50	45.60
<i>Salvinia</i> and <i>Brotia</i>	0.00	10.00	12.50	15.00
Soybean meal	22.80	20.40	19.60	19.10
Oil	0.60	1.00	0.70	1.00
Brand	13.90	7.00	9.10	8.60
Fish meal	7.00	7.00	6.80	6.80
Lime	0.60	0.40	0.30	0.30
Premix	0.60	0.30	0.30	0.30
Methionine	0.40	0.30	0.30	0.30
Lysine	0.50	0.50	0.40	0.50
Total	100.00	100.00	100.00	100.00
Nutrition content				
Energy metabolism	2922.96	2904.27	2900.42	2900.139
Crude protein	22.09	22.02	22.03	22.01
Crude fat	4.40	4.15	4.18	4.41
Crude fiber	5.67	8.70	8.55	9.11
Methionine	0.76	0.66	0.65	0.65
Lysine	1.43	1.39	1.28	1.36
Arginine	0.54	1.28	1.27	1.25
Ca (%)	1.20	1.15	1.27	1.34
P (%)	0.76	0.72	0.70	0.70

T₀-T₄: Treatment with FSM/NFSM, T₀: Basal diet, T₁, T₂, T₃ and T₄: 15% NFSM, 15% FSM, 17.5% FSM and 20% FSM, respectively, Energy metabolism (EM) was measured based on the Balton formula according to Anggorodi (1994), Nitrogen free extract = 100-(% water+% Ash+% Crude protein+% Crude fat+% Crude fiber), Energy metabolism (EM) = 40.81 {0.87 (Crude protein+2.25 Crude fat+Nitrogen free extract+4.9)}

with 584.4 g of warm mineral water and mineral. The mixing formula was spread onto the tray and then covered by thin paper and incubated for one week¹¹.

Experimental birds: The study was carried out according to the guidelines for applied nutrition experiments in poultry¹². Starter periods of 5 week-old 120 Pengging ducks with an average body weight of 734.25±0.52 g were maintained until they were 12 weeks old. The birds were housed in groups under standard conditions in a 20 unit postal cage with 4 individuals/unit. The ducks were fed 3 times/day ad libitum with 2,800 kcal of a metabolic energy and 18% crude protein as described in Table 1. Their fattening duration was 49 days. Individuals were chosen for the experiment by a completely randomized design with 4 treatment groups and 6 replicates. Treatments were: T₀ (basal diet), T₁ (10% SB), T₂ (12.5% SB) and T₃ (15% SB).

Preparation of egg yolk for analyses: The eggs used for analyses were prepared according to the procedure described by AOAC¹³ and El-Sayed *et al.*¹⁴. The eggs were first hard-cooked and allowed to cool, after which the weight of the boiled egg was recorded. The egg shell was peeled off and

weighed followed by the careful removal of the egg white (albumen). The yolks were separated, weighed and crumbled. A one gram sample of each yolk was homogenized with 15 mL of chloroform-methanol 2:1 (v/v) and thoroughly mixed and filtered. Egg homogenized filtrates were designated as egg yolk samples.

Chemical analyses: Total cholesterol, HDL-cholesterol and total triglyceride concentrations of the egg yolks were determined using the respective RANDOX® cholesterol assay kit from the Randox Laboratories United Kingdom. The kit contained cholesterol assay reagent and standard cholesterol solution used for the calibration curve.

Total cholesterol: Ten microlitter of egg yolk sample and 10 µL of deionized water were pipetted into their respective test tubes. This was followed by 1000 µL of total cholesterol assay reagent. The test tubes were incubated for 15 min at 37°C, after which the absorbance of the sample was read at 500 nm against the reagent blank. The various cholesterol concentrations, recorded as mg g⁻¹ of egg yolk, were computed from the values obtained from the various cholesterol standard curves.

HDL-cholesterol: The HDL-cholesterol assay kit (RANDOX®) contained a cholesterol precipitant. First, 500 µL of egg yolk sample was pipetted into a test tube and 1000 µL of the precipitant was added, mixed and then centrifuged for 15 min. After centrifuging, 100 µL of the supernatant and 100 µL of deionized water were pipetted into their respective test tubes. This was followed by the addition of 1000 µL of HDL-cholesterol reagent. The test tubes were incubated for 15 min at 37°C, after which the absorbance of the sample and standard, read at 500 nm and the computation of HDL-cholesterol concentrations of the egg yolk were done as described for total cholesterol^{15,16}.

Total triglyceride: The total triglyceride assay kit contained a buffer solution, an enzyme reagent and a standard triglyceride solution. The working enzyme reagent was constituted by adding 15 mL of buffer to the enzyme reagent. The triglyceride assay was performed as previously described except that test tubes were incubated for 15 min at room temperature.

LDL-cholesterol: LDL-cholesterol was estimated using the Friedewald equation¹⁷ as follows:

$$\text{LDL-cholesterol} = \text{Total cholesterol} - \text{Triglyceride} - (\text{HDL-cholesterol})$$

Feed conversion ratio (FCR): Feed conversion ratio was calculated based on a comparison between the rations consumed by the resulting weight gain with time and the same unit of body weight¹⁸:

$$FCR = \frac{\text{Amount of feed consumed}}{\text{Body weight gain}}$$

Feed costs: Feed costs were calculated from the 4-10 weeks of the experiment's duration. Feed cost was based on the price of feed kg⁻¹ multiplied by the number of daily feedings and expressed in IDR/head/day¹⁹. Feed price is obtained by multiplying the price of each material multiplied by the number of diet constituents and expressed as IDR kg⁻¹, while the price of SB is obtained through the calculation of wages divided by the number of person-days generating SM in its wet form, which is then converted into the dry ingredients plus the cost of transportation and milling to a powder form calculated as IDR kg⁻¹.

Income over feed cost (IOFC): Income over feed cost is obtained by the difference between income and the costs of the feed and expressed in IDR/head. Income over feed cost was calculated based on the Eq:

$$IOFC = \left[\frac{(\text{Body weight} \times \text{Price of chicken kg}^{-1} \text{ live}) - (\Sigma \times \text{feed intake of feed cost kg}^{-1})}{\text{}} \right]$$

RESULTS AND DISCUSSION

The effects of supplementation with *Salvinia-Brotia* (SB) in the feed (Table 1) on the weight of duck eggs and some physical parameters are shown in Table 2. In this 8 weeks experimental trial it was observed that supplementation at the test level does not significantly affect the body weight of the ducks or physical characteristics of the eggs.

Table 3 shows the effect of SB supplementation in duck feed on the total cholesterol, LDL, HDL and triglycerides. In all the parameters related to cholesterol, significant differences

were found in egg yolks between the treatment groups compared to the control group. The addition of SB at various percentages was shown to reduce total cholesterol and triglycerides in the yolk compared to the control diet. The addition of 12.5% SB (T₂) reduced the cholesterol compared to the 10% SB (T₁), but did not differ significantly with the addition of 15% SB (T₃). Furthermore, SB supplementation significantly lowered the LDL content in egg yolks and 12.5% SB (T₂) resulted in the lowest LDL and triglyceride contents among the treatments, accompanied by increasing contents of HDL.

Most people have realized that duck eggs are more delicious than chicken eggs because of their higher fat content²⁰. Various studies suggested that the proportion of unsaturated fatty acids were the highest among the various fatty acids in the total fat of duck eggs^{10,21,22}. That's why, duck eggs are a source of healthy fat in the daily diet. The interesting thing revealed in this study is that the use of SB as a feed additive not only enhances both the productivity of the livestock but also the quality of their products. These results indicate that the supplementation of SB in the rations significantly changed some of the parameters associated with the egg yolk lipid profile, including total cholesterol, LDL, HDL and triglycerides (Table 3).

It is generally understood that the quality of the feed can affect the chemical composition of egg fat. Enriching the feed with crude fiber of plant origin can reduce the absorption of glucose from the gut so that blood insulin levels remain low. This results in elevated glucagon levels to trigger lipolysis in the network²³.

As shown as in Table 4, the finisher weights of ducks fed T₂ were the best, followed by T₀, T₁ and T₃, respectively. The

Table 2: Duck weight and some characteristic performance of duck

Items	T ₀	T ₁	T ₂	T ₃
Duck weight (kg)	1.92 ± 0.03	1.82 ± 0.02	1.81 ± 0.10	1.89 ± 0.09
Egg weight (g)	63.25 ± 0.76	60.92 ± 0.01	59.45 ± 0.15	62.81 ± 0.11
Yolk weight (g)	40.66 ± 0.04	39.92 ± 0.06	41.92 ± 0.21	38.98 ± 0.17
Eggshell weight (g)	6.12 ± 0.03	5.56 ± 0.03	5.50 ± 0.06	6.34 ± 0.07

T₀-T₃: Treatments, T₀: Control ration, T₁, T₂ and T₃: SB 10% SB, 12,5% SB, 15% SB, respectively

Table 3: Duck yolk egg cholesterol supplemented by SB

Composition	T ₀	T ₁	T ₂	T ₃
Crude protein (%)	28.61 ± 3.11	28.64 ± 0.02	28.71 ± 0.15	29.16 ± 0.08
Crude fat (%)	59.90 ± 1.76	59.78 ± 2.01	58.40 ± 0.15	57.08 ± 1.11
Total cholesterol	53.93 ± 2.19 ^a	50.86 ± 1.72 ^{ab}	42.32 ± 1.71 ^c	46.41 ± 3.69 ^{bc}
LDL	45.27 ± 0.81 ^a	41.93 ± 1.23 ^a	31.22 ± 0.89 ^b	36.09 ± 0.97 ^{ab}
HDL	8.65 ± 0.51 ^b	8.93 ± 1.16 ^b	11.10 ± 0.64 ^a	10.31 ± 0.81 ^{ab}
Total triglycerides	411.56 ± 12.00 ^a	399.29 ± 9.60 ^{ab}	338.03 ± 7.00 ^c	354.40 ± 23.76 ^{bc}

^{a, b, c}Means in the same row with different superscripts differ significantly (p<0.05) according to Duncan's multiple range test. SB: *Salvinia-Brotia*, T₀-T₃: Treatments, T₀: Control ration, T₁, T₂ and T₃: SS 10% SS, 12,5% SS, 15% SS, respectively

Table 4: Weight gain, FCR, finisher weight, IOFC of ducks fed by SB

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Weight gain (g)	925.77 ^a	865.80 ^a	940.60 ^a	820.60 ^a
FCR	7.22 ^a	7.40 ^a	7.40 ^a	8.12 ^a
Finisher weight (g)	1640.22 ^a	1575.30 ^b	1702.30 ^a	1560.50 ^b
Feed cost (IDR)	29648.26 ^a	26792.26 ^b	23153.90 ^c	33526.90 ^a
IOFC (IDR)	19551.07 ^a	20457.56 ^a	27906.14 ^b	13273.33 ^c

^{a,b,c}Means in the same row with different superscripts differ significantly ($p < 0.05$) according to Duncan's multiple range test

IOFC of ducks fed T₂ gave the best performances compared with the other rations. The weight gain in ducks did not differ significantly among the treatments. Application of SB 12.5% on duck feed gave implication economic could increase income over feed cost and reduce feed cost.

CONCLUSION

The incorporation of *Salvinia molesta* and *Brotia costula* by upto 12.5% in the ducks' diet gave the best profitability in the broiler rearing system. The incorporation of 12.5% of *Salvinia molesta* and *Brotia costula* in the duck feed would reduce the total cholesterol, feed cost and could increase the IOFC.

ACKNOWLEDGMENT

This study was supported by internal funding from the Faculty of Animal and Agricultural Sciences (PNBP 2017).

REFERENCES

- Adzitey, F. and S.P. Adzitey, 2011. Duck production: Has a potential to reduce poverty among rural households in Asian communities: A review. *J. World's Poult. Res.*, 1: 7-10.
- Anonymous, 2013. Populasi dan produksi peternakan di Indonesia. [General directory of animal husbandry 2013. Population and production of farm animal in Indonesia]. <http://www.pertanian.go.id/Indikator/tabel-4-pop-prod-nak.pdf>
- Frobose, H.L., R.C. Sulabo, J.M. DeRouchey, D. Ryder and M.D. Tokach *et al.*, 2014. The effects of diet blending and feed budgeting on finishing pig growth performance, carcass characteristics and economic return. *Prof. Anim. Scient.*, 30: 375-392.
- Setiadi, A., S.I. Santoso, Sumarsono, L.D. Mahfudz and A.B. Susanto, 2016. An economic analysis of kampung chicken production using the small water plant *Azolla microphylla* in their feed. *Pak. J. Nutr.*, 15: 264-267.
- Santoso, S.I. and A. Setiadi, 2016. Profitable utilization of giant *Salvinia*, *Salvinia molesta*, as local duck feed. *Int. J. Poult. Sci.*, 15: 121-125.
- Dwiloka, B., A. Setiadi, S.I. Santoso, E. Suprijatna and S. Susanti, 2015. Effects of duck feed supplemented with invasive giant *Salvinia* (*Salvinia molesta*) on duck meat characteristics. *Turk. J. Vet. Anim. Sci.*, 39: 668-675.
- Alamsjah, M.A. and Prayogo, 2014. Mineral nutrient content from *Gracilaria* sp. waste as biofertilizer on intensive aquaculture with aquaponic system. *J. Nat. Sci. Res.*, 4: 65-74.
- Tang, J.C., H. Taniguchi, H. Chu, Q. Zhou and S. Nagata, 2009. Isolation and characterization of alginate-degrading bacteria for disposal of seaweed wastes. *Lett. Applied Microbiol.*, 48: 38-43.
- Ge, L., P. Wang and H. Mou, 2011. Study on saccharification techniques of seaweed wastes for the transformation of ethanol. *Renewable Energy*, 36: 84-89.
- Baeza, E., M.R. Salichon, G. Marche, N. Wacrenier, B. Dominguez and J. Culioli, 2000. Effects of age and sex on the structural, chemical and technological characteristics of mule duck meat. *Br. Poult. Sci.*, 41: 300-307.
- Mensor, L.L., F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. dos Santos, C.S. Coube and S.G. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.*, 15: 127-130.
- Reddy, D.V., 2001. Applied Nutrition: Livestock, Poultry, Pets, Rabbits and Laboratory Animals. 2nd Edn., Oxford and IBH-Pubs Company, New Delhi, India.
- AOAC., 1999. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Arlington, Virginia, USA.
- El-Sayed, W.M., W.M. Hussin, A.A. Mahmoud and M.A. AlFredan, 2013. The *Conyza triloba* extracts with high chlorophyll content and free radical scavenging activity had anticancer activity in cell lines. *Biomed. Res. Int.* 10.1155/2013/945638.
- Olmedilla-Alonso, B., F. Jimenez-Colmenero and F.J. Sanchez-Muniz, 2013. Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Sci.*, 95: 919-930.
- Dawson, B. and R.G. Trapp, 2001. Basic and Clinical Biostatistics. Lange Medical Books/McGrawHill Medical Publishing Division, New York, USA.
- Fasseas, M.K., K.C. Mountzouris, P.A. Tarantilis, M. Polissiou and G. Zervas, 2008. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chem.*, 106: 1188-1194.
- Wang, Y.Z., Z.R. Xu and J. Feng, 2004. The effect of betaine and DL-methionine on growth performance and carcass characteristics in meat ducks. *Anim. Feed Sci. Technol.*, 116: 151-159.
- Poon, K. and A. Weersink, 2011. Factors affecting variability in farm and off-farm income. *Agric. Finance Rev.*, 71: 379-397.

20. Ali, M.S., G.H. Kang, H.S. Yang, J.Y. Jeong, Y.H. Hwang, G.B. Park and S.T. Joo, 2007. A comparison of meat characteristics between duck and chicken breast. *Asian-Aust. J. Anim. Sci.*, 20: 1002-1006.
21. Baeza, E., C. Dessay, N. Wacrenier, G. Marche and A. Listrat, 2002. Effect of selection for improved body weight and composition on muscle and meat characteristics in Muscovy duck. *Br. Poult. Sci.*, 43: 560-568.
22. Cobos, A., A. Veiga and O. Diaz, 2000. Chemical and fatty acid composition of meat and liver of wild ducks (*Anas platyrhynchos*). *Food Chem.*, 68: 77-79.
23. Reimer, R.A., A.D. Maurer, L.K. Eller, M.C. Hallam, R. Shaykhutdinov, H.J. Vogel and A.M. Weljie, 2012. Satiety hormone and metabolomic response to an intermittent high energy diet differs in rats consuming long-term diets high in protein or prebiotic fiber. *J. Proteome Res.*, 11: 4065-4074.