

ISSN 1682-8356  
ansinet.org/ijps



INTERNATIONAL JOURNAL OF  
**POULTRY SCIENCE**

**ANSI***net*

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

## Performance and Carcass Characteristics of Japanese Quails Fed Diets Containing Wolffia Meal [*Wolffia globosa* (L). Wimm.] as a Protein Replacement for Soybean Meal

A. Chantiratikul<sup>1</sup>, P. Chantiratikul<sup>2</sup>, A. Sangdee<sup>3</sup>, U. Maneechote<sup>4</sup>, C. Bunchasak<sup>5</sup> and O. Chinrasri<sup>1</sup>

<sup>1</sup>Animal Feed Resources and Animal Nutrition Research Unit, Faculty of Veterinary and Animal Sciences, Mahasarakham University, Muang, Maha Sarakham, 44000, Thailand

<sup>2</sup>Department of Chemistry and Center of Innovation in Chemistry (PERCH-CIC)

<sup>3</sup>Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand

<sup>4</sup>Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sansai, Chiang Mai, 50290, Thailand

<sup>5</sup>Department of Animal Science, Faculty of Agriculture, Kasetsart University, Jatujak, Bangkok, 10900, Thailand

**Abstract:** The objectives of this study were to determine the effect of replacement of Crude Protein (CP) from Soybean Meal (SBM) with CP from Wolffia meal [*Wolffia globosa* (L). Wimm.] on performance and carcass quality in Japanese quails. Using Completely Randomize Design (CRD), two hundred eighty-eight quails were divided into four treatments. Each treatment consisted of six replicates and each replicate contained twelve quails. The dietary treatments were T<sub>1</sub>: control diet, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>: CP from SBM was replaced by CP from Wolffia meal at 25, 50 and 75%, respectively. The results showed that the feed intake of quails significantly decreased ( $p < 0.05$ ) when over 50% of CP from SBM was replaced by CP from Wolffia meal in the diet. However, replacement of CP from SBM with CP from Wolffia meal in the diets did not alter ( $p > 0.05$ ) feed efficiency, performance and carcass quality of quails. Skin pigmentation increased ( $p < 0.05$ ) with increasing CP replacement from Wolffia meal. In conclusion, 50% of CP from Wolffia meal could appropriately replace CP from SBM in the diet of quail.

**Key words:** Duckweed, performance, carcass quality, skin pigmentation, quails

### INTRODUCTION

The main cost of animal production is cost of feed, which is generally associated with high cost of important protein concentrates, Soybean Meal (SBM) and fish meal (Teguia and Fon Fru, 2007). Therefore, using local protein sources to replace the imported protein sources is one solution to reduce production costs. Wolffia meal (*Wolffia* spp.), one of the duckweed species, belongs to the botanical family *Lemnaceae*. The family consists of five genera *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella* with over forty identified species (Les *et al.*, 2002). Wolffia meal is a small circular floating weed about 0.5-1.5 mm in length that lives in tropical and subtropical lakes and marshes (Landolt, 1986). It has been used as a vegetable in the Indochinese peninsular for many generations (Bhanthumnavin and McGarry, 1971). Under ideal growth conditions of optimal nutrient and sunlight availabilities and water temperature Wolffia meal is capable of doubling its biomass in 16 h to 4 days (Reid, 2004). Dry matter yield from commercial-scale cultivation of *Lemna*, *Spirodela* and Wolffia

species in Bangladesh ranges from 13-38 tons/ha/year, which is a rate exceeding single-crop soybean production six to tenfold (Skillicorn *et al.*, 1993). Furthermore, Crude Protein (CP) content (30-45%) (Rusoff *et al.*, 1980; Skillicorn *et al.*, 1993) and amino acid profile (Rusoff *et al.*, 1980) of Wolffia meal were comparable to those of SBM. The above information obviously reflects that Wolffia meal is a promising alternative protein source in animal diets.

Utilization of duckweed species, namely, *Lemna gibba*, *Lemna perpusilla*, *Spirodela punctata* and *Lemna minor* as a protein source, have been studied in domestic animals including cattle (Huque *et al.*, 1996; Chewewattanagool, 2002), goat (Reid, 2004), sheep (Damry *et al.*, 2001), swine (Leng *et al.*, 1995; Gutierrez *et al.*, 2001; Dung *et al.*, 2002), ducks (Men *et al.*, 2001; Men *et al.*, 2002; Khandaker *et al.*, 2007), broilers (Sokantat, 1990; Islam *et al.*, 1997; Hausteine *et al.*, 1992; Hausteine *et al.*, 1994), layers (Hausteine *et al.*, 1990) and quails (Sokantat, 1990). However, using Wolffia meal in animal diet has been only reported in laying hens (Hausteine *et al.*, 1990; Chantiratikul *et al.*, 2010). The

information of utilization of Wolffia meal as protein source in animal diet is very scarce. Therefore, the present study evaluated productive performance and carcass traits of Japanese quails fed diets containing various CP replacement levels of Wolffia meal (*Wolffia globosa* (L) Wimm., accession number GQ221774).

## MATERIALS AND METHODS

The experiment was conducted under appropriate animal care regulations. Two hundred eighty eight Japanese quails were obtained at 1 d of age and fed control diet until 7 d of age. Afterwards, quails were randomly assigned to 4 groups, with 6 replicates of 12 quails each according to Completely Randomized Design (CRD). Vaccinations for Newcastle disease and infectious bronchitis were applied to quails at 7 d and 14 d of ages. The experiment was conducted in evaporative system housing. Internal light was provided for 24 h/d. The room temperature was 32°C at the first week and was gradually decreased to 25°C from d 14 until the end of the trial.

Fresh Wolffia meal (*Wolffia globosa* (L). Wimm.) was purchased from a local producer, who cultivated Wolffia meal as human food and dried under sunlight for 1-2 days. Dried Wolffia meal was ground through 2 mm. screen and stored in air tight bags. The chemical composition of used Wolffia meal was previously reported by Chantiratikul *et al.* (2010).

The dietary treatments were T1 = control diet; T2, T3 and T4 = 25, 50 and 75% of the CP from SBM was replaced

by the CP of Wolffia meal, respectively. All diets were isonitrogenous and isocaloric and formulated to meet the nutrient requirement of quails according to NRC (1994) as presented in Table 1 and 2. The used Metabolizable Energy (ME) value of Wolffia meal for dietary treatment formulation was 1047.80 kcal/kg (Poonpanpan *et al.*, 2009). The quails received diets and water for *ad libitum* consumption during the entire experimental period.

The dietary treatments were randomly collected at the end of each week for determination of chemical composition (AOAC, 1999). Feed consumption was recorded daily. Body weight was determined at the beginning and the end of the experiment. Feed Conversion Ratio (FCR) and Protein Efficiency Ration (PER) were estimated as kilograms of feed consumed per kilogram of gain and as grams of weight gain per gram of protein intake, respectively. The mortality of quails was recorded during the entire experiment.

On d 42, 24 quails from each treatment were individually weighed live, stunned and slaughtered by neck cutting and exsanguinated. Carcasses were plucked, eviscerated and weighed. Breast skin color was measured using the Kemin colour fan (Kemin Industries, Des Moines, IA), with minimum and maximum values ranging from 1-15. Afterwards, carcasses were processed by removing wings, skinless breast muscle and legs. All separated components were later weighed.

Table 1: Feed ingredient and chemical composition of starter diets

Ingredients (%)	Dietary treatments <sup>1</sup>			
	T1	T2	T3	T4
Corn	44.07	44.07	44.07	43.24
Rice bran	8.73	5.71	2.66	0.00
Full fat soybean	14.00	14.00	14.00	14.00
Soybean meal (44%CP)	24.00	18.00	12.00	6.00
Wolffia meal (29.6%CP)	0.00	8.92	17.84	26.76
Fish meal	6.00	6.00	6.00	6.00
DL-methionine	0.07	0.07	0.07	0.07
Soybean oil	1.40	1.50	1.66	2.20
Dicalcium phosphate	0.00	0.10	0.16	0.30
Oyster shell	1.23	1.13	1.04	0.93
Sodium chloride	0.25	0.25	0.25	0.25
Premix <sup>2</sup>	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<b>Nutrient composition (%)</b>				
Dry matter	93.61	94.16	94.36	94.66
Crude protein	24.08	24.08	24.08	24.01
Ether extract	5.81	4.92	5.03	6.03
Crude fiber	2.71	2.89	3.41	4.27
Ash	6.51	7.50	8.37	9.63
Metabolizable energy <sup>3</sup> (kcal/kg)	2,906.45	2,900.90	2,900.38	2,903.85

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>2</sup>Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D<sub>3</sub>, 11 mg vitamin E, 1.5 mg vitamin K<sub>3</sub>, 1.5 mg thiamin, 4 mg riboflavin, 10 mg pantothenic acid, 0.4 folic acid, 4 mg pyridoxine, 22 mg niacin, 0.4 mg colabamin, 0.1 mg biotin, 60 mg Fe, 70 mg Mn, 50 mg Zn, 8 mg Cu, 0.5 mg Co, 0.7 mg I, 0.1 mg Se. <sup>3</sup>Calculated value

Table 2: Feed ingredient and chemical composition of grower diets

Ingredients (%)	Dietary treatments <sup>1</sup>			
	T1	T2	T3	T4
Corn	51.20	48.19	44.30	38.52
Rice bran	8.00	4.04	1.00	0.00
Full fat soybean	11.36	13.45	15.4	17.08
Soybean meal (44%CP)	24.00	18.00	12.00	6.00
Wolffia meal (29.6%CP)	0.00	8.92	17.84	26.76
Fish meal	3.00	3.00	3.00	3.00
DL-methionine	0.13	0.13	0.13	0.13
Soybean oil	0.00	1.96	4.00	6.18
Dicalcium phosphate	0.50	0.60	0.72	0.83
Oyster shell	1.23	1.13	1.03	0.92
Sodium chloride	0.25	0.25	0.25	0.25
Premix <sup>2</sup>	0.25	0.25	0.25	0.25
L-Lysine	0.08	0.08	0.08	0.08
Total	100.00	100.00	100.00	100.00
<b>Nutrient composition (%)</b>				
Dry matter	93.39	93.84	94.46	94.78
Crude protein	22.45	22.23	21.52	21.53
Ether extract	5.21	6.13	8.94	9.11
Crude fiber	2.91	3.55	4.58	4.92
Ash	6.57	7.38	8.64	9.42
Metabolizable energy <sup>3</sup> (kcal/kg)	2,941.08	2,941.34	2,941.62	2,942.18

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>2</sup>Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D<sub>3</sub>, 11 mg vitamin E, 1.5 mg vitamin K<sub>3</sub>, 1.5 mg thiamin, 4 mg riboflavin, 10 mg pantothenic acid, 0.4 folic acid, 4 mg pyridoxine, 22 mg niacin, 0.4 mg colabamin, 0.1 mg biotin, 60 mg Fe, 70 mg Mn, 50 mg Zn, 8 mg Cu, 0.5 mg Co, 0.7 mg I, 0.1 mg Se. <sup>3</sup>Calculated value

**Statistical analysis:** The data of productive performance and carcass characteristics were analyzed by analysis of variance technique appropriate for Completely Randomized Design (SAS, 1996). The model used was:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where:

$Y_{ij}$  = Observation,

$\mu$  = Population mean,

$T_i$  = Diet effect (i = 1 to 4) and

$\epsilon_{ij}$  = Residual error

The differences among means of each parameter were compared by Duncan's New Multiple Range Test (Steel and Torries, 1980). A probability level of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

Feed intake of quails significantly decreased ( $p < 0.05$ ) when over 50% of CP from SBM was replaced by CP from Wolffia meal in the diet. However, replacement of CP from SBM with CP from Wolffia meal in the diets did not alter ( $p > 0.05$ ) average daily gain, FCR, PER and mortality of quails (Table 3).

Carcass dressing and carcass characteristics of quails were not significantly affected ( $p > 0.05$ ) by dietary replacement of CP from SBM with CP from Wolffia meal (Table 4).

Breast skin color increased ( $p < 0.05$ ) with increasing CP replacement from Wolffia meal in the diet (Table 4).

## DISCUSSION

The small Wolffia meal cultivation farms have been recently commercialized in northeastern Thailand. The cultivated Wolffia meal contained 29.61% of CP and was successfully tested as protein replacement for SBM in diets of laying hens (Chantiratikul *et al.*, 2010). Presently, there is no available information using Wolffia meal as protein replacement in the diet of quails. This was the first study on utilization of cultivated Wolffia meal as a protein replacement for SBM in diets of quails.

Although, productive performance and carcass quality of quails in this study were not different ( $p > 0.05$ ) among experimental groups, decreased feed intake was found in quails fed diets containing over 50% of CP replacement from Wolffia meal or over 9% of Wolffia meal (Table 3, 4). This result is in accordance with previous studies (Jhori and Sharma, 1979; Islam *et al.*, 1997) which reported that dried *Lemna minor* could be used in broiler ration at a level of 9-10% without affecting weight gain, feed efficiency and performance.

However, Sokantat (1990) found that 10-20% of CP from SBM could be replaced by CP from *Lemna* spp. in quail diets (12% of dietary *Lemna* spp.) without adverse effect on intake, weight gain, feed efficiency and carcass dressing percentage. Additionally, the studies in broilers revealed that feed intake and productive performance of chicks decreased ( $p < 0.05$ ) when the level of dietary *Lemna gibba* rose above 15% (Haustein *et al.*, 1992; 1994). The above suggested inclusion levels of dietary *Lemna* spp. (12 and 15% in the diets of quail and

Table 3: Effect of replacing protein from soybean meal with protein from Wolffia meal on feed efficiency and productive performance in quails

Items	Dietary treatments <sup>1</sup>				SEM
	T1	T2	T3	T4	
Feed intake (g/day)	12.08 <sup>a</sup>	12.17 <sup>a</sup>	11.72 <sup>ab</sup>	11.41 <sup>b</sup>	0.11
Average daily gain (g/day)	3.32	3.32	3.24	3.08	0.04
Feed conversion ratio (feed/gain)	3.64	3.67	3.62	3.71	0.03
Protein efficiency ratio	1.21	1.21	1.22	1.19	0.01
Mortality rate (%)	2.78	2.78	2.78	4.17	0.84

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>ab</sup>Means in the same row with different superscripts are significantly different (p<0.05)

Table 4: Effects of replacement of protein from soybean meal with protein from Wolffia meal on carcass dressing, carcass characteristics and breast skin colour in quails

Items	Dietary treatments <sup>1</sup>				SEM
	T1	T2	T3	T4	
Carcass dressing (% BW)	79.17	74.49	76.99	76.45	1.06
<b>Carcass characteristics (% carcass weight)</b>					
Wings	7.00	6.56	6.94	6.80	0.08
Legs	18.35	17.27	16.97	17.39	0.20
Breast	21.99	20.63	20.42	20.23	0.28
Abdominal fat	0.43	0.48	0.47	0.53	0.05
Breast skin colour	2.12 <sup>d</sup>	2.54 <sup>c</sup>	3.71 <sup>b</sup>	4.62 <sup>a</sup>	0.21

<sup>1</sup>T1 = control; T2, T3 and T4 = 25, 50 and 75% CP from SBM were replaced by CP from Wolffia meal, respectively.

<sup>abcd</sup>Means in the same row with different superscripts are significantly different (p<0.05)

broiler respectively) were slightly higher than that (9% in the diets of quail) of Wolffia meal in the current report. The different outcome occurred probably due to duckweed species and variation of their chemical composition.

Generally, passage rate and feed intake changed based on type of feed used in formulating diets (Isshihi and Nakahiro, 1989). Therefore, the phenomenon of decreased feed intake of quails could be explained by both physical form of Wolffia meal i.e. dustiness and loose in texture and higher dietary fiber (Table 1, 2) with increasing CP replacement from Wolffia meal.

Increased skin pigmentation of quails fed dietary Wolffia meal in the current study is in agreement with the previous study in quails using *Lemna* spp. (Sokantat, 1990) and in broilers using *Lemna gibba* (Haustein *et al.*, 1994). Furthermore, duckweed has been reported to increase pigmentation in yolk of laying hens (Haustein *et al.*, 1990; Nolan *et al.*, 1997; Chantiratikul *et al.*, 2010). Those results indicated that Wolffia meal and other duckweed species were promising sources of pigments for poultry.

**Conclusion:** Dietary replacement of CP from SBM with CP from Wolffia meal over 50% decreased (p<0.05) feed intake with no effect on productive performance and carcass quality in quails. However, skin pigmentation increased with increasing CP replacement from Wolffia meal. The optimal CP replacement level from Wolffia meal for SBM was 50% in the diets of quail.

## ACKNOWLEDGEMENTS

This research was funded by Thailand Research Fund (TRF) and Commission on Higher Education (CHE).

## REFERENCES

- AOAC, 1999. Official Methods of Analysis. 16th Edn., Association of Official Analysis Chemists, Washington, DC.
- Bhanthumnavin, K. and M. McGarry, 1971. *Wolffia arrhiza* as a possible source of inexpensive protein. *Nature*, 232: 485.
- Chantiratikul, A., O. Chinrasri, P. Chantiratikul, A. Sangdee, U. Maneechote and C. Bunchasak, 2010. Effect of replacement of protein from soybean meal with protein from Wolffia meal (*Wolffia globosa* (L.) Wimm.) on performance and egg production in laying hens. *Int. J. Poult. Sci.*, 9: 283-287.
- Chewewattanagool, S., 2002. The Use of Duckweed Protein Replaced Soybean Meal Protein in Crossbred Dairy (Holstein Friesian) Cattle Fed Cassava-Based Concentrate. Master Thesis in Animal Science. Khon Kean University. Khon Kean, Thailand.
- Damry, H., J.V. Nolan, R.E. Bell and E.S. Thomson, 2001. Duckweed as a protein source for fine-wool Merino sheep; Its edibility and effects on wool yield and characteristics. *Asian-Aust. J. Anim. Sci.*, 14: 507-514.
- Dung, N.N.X., L.H. Manh and P. Uden, 2002. Tropical fibre sources for pigs digestibility, digesta retention and estimation of fibre digestibility *in vitro*. *Anim. Feed Sci. Technol.*, 102: 109-124.

- Gutierrez, K., L. Sangines, F. Perez and L. Marinez, 2001. Studies on the potential of the aquatic plant *Lemna gibba* for pig feeding. Cuban J. Agric. Sci., 35: 343-348.
- Haustein, A.T., R.H. Gillman, P.W. Skillicorn, V. Vergara, V. Guevara and A. Gastanaduy, 1990. Duckweed, a useful strategy for feeding chickens: Performance of layers fed with sewage-grown *Lemnaceae* species. Poult. Sci., 69: 1835-1844.
- Haustein, A.T., R.H. Gillman, P.W. Skillicorn, V. Guevara, F. Diaz, V. Vergara, A. Gastanaduy and J.B. Gilman, 1992. Compensatory growth in broiler chicks fed on *Lemna gibba*. Br. J. Nutr., 68: 329-335.
- Haustein, A.T., R.H. Gillman, P.W. Skillicorn, H. Hanna, F. Diaz, V. Guevara, V. Vergara, A. Gastanaduy and J.B. Gilman, 1994. Performance of broiler chickens fed diets containing duckweed. J. Agric. Sci., 122: 285-289.
- Huque, K.S., S.A. Chowdhury and S.S. Kibria, 1996. Study on the potentiality of duckweeds as a feed for cattle. Asian-Aust. J. Anim. Sci., 9: 133-137.
- Islam, K.M.S., Shakjalal, A.M.M. Traeque and M.A.R. Howlider, 1997. Complete replacement of dietary fish meal by duckweed and soybean meal on the performance of broilers. Asian-Aust. J. Anim. Sci., 10: 629-634.
- Isshihi, Y. and Y. Nakahiro, 1989. Feed digestibility in different intestinal parts of chicks. Japanese J. Zootech. Sci., 60: 1082-1086.
- Jhori, T.S. and P.N. Sharma, 1979. Studies on utilization of dried duckweed (*Lemna minor*) in chicks. In. J. Poult. Sci., 14 (suppl): 14.
- Khandaker, T., Md.J. Khan, Md. Shahjalal and Md.M. Rahman, 2007. Use of duckweed (*Lemna perpusilla*) as a protein source feed item in the diet of semi-scavenging Jinding layer ducks. J. Poult. Sci., 44: 314-321.
- Landolt, E., 1986. The family of Lemnaceae-a monographic study. Vol. 1 Veröffentlichungen des Geobotanischen Institutes der ETH. Stiftung Rober Zurich, 71: 566.
- Leng, R.A., J.H. Stambolie and R. Bell, 1995. Duckweed-a potential high protein feed resource for domestic animals and fish. Livest. Res. Rural. Develop. 7. Available: <http://www.cipav.org.co/lrrd/lrrd7/1/3.htm>. Accessed Feb 18, 2010.
- Les, D.H., D.J. Crawford, E. Landolt, J.D. Gabel and R.T. Kimball, 2002. Phylogeny and systematics of Lemnaceae, the duckweed family. Systematic Botany, 27: 221-240.
- Men, B.X., B. Ogle and J.E. Lindberg, 2001. Use of duckweed as a protein supplement for growing ducks. Asian-Aust. J. Anim. Sci., 14: 1741-1746.
- Men, B.X., B. Ogle and J.E. Lindberg, 2002. Use of duckweed as a protein supplement for breeding ducks. Asian-Aust. J. Anim. Sci., 15: 866-871.
- National Research Council, 1994. Nutrition Requirement of Poultry, 9th Edn., National Academy Press, Washington, DC.
- Nolan, J.V., R.E. Bell, E. Thomson, D. Bremner and W. Ball, 1997. Duckweed (*Spirodela punctata*) as a protein and pigment source in diets for layers. Proc. Aust. Poult. Sci. Symp., 9: 166-170.
- Poonpan, P., A. Chantiratikul, O. Chinrasri and S. Santhaweesuk, 2009. Evaluation of metabolizable energy of *Wolffia* meal (*Wolffia globosa* (L.) Wimm.) in broilers. The 2nd International Conference on Sustainable Animal Agriculture for Developing Countries. 8<sup>th</sup>-11<sup>th</sup> November 2009. Kuala Lumpur, Malaysia, pp: 229-230.
- Reid, W.S.Jr., 2004. Exploring Duckweed (*Lemna gibba*) As a Protein Supplement for Ruminant Using the Boer Goat (*Capra Hircus*) As a Model. Master Thesis. North Carolina State University, North Carolina, USA.
- Rusoff, L., E.W. Blakeney Jr. and D.D. Culley Jr., 1980. Duckweeds (*Lamnaceae* Family): A potential source of protein and amino acids. J. Agric. Food Chem., 28: 848-850.
- SAS, 1996. SAS/STAT® User's Guide (Release 6.03 ed.). SAS Inst. Inc. Cary, NC.
- Skillicorn, P., W. Spira and W. Journey, 1993. Duckweed aquaculture - a new aquatic farming system for developing countries. The World Bank, Washington, DC.
- Sokantat, D., 1990. The use of duckweed as a substituted protein source for soybean meal in broiler and Japanese quail diets. M.Sc. Thesis in Animal Science. Khon Kaen University, Khon Kaen.
- Steel, R.G.D. and J.H. Torries, 1980. Principle and Procedure of Statistic a Biometrical Approach. 2nd Edn., McGraw-Hill, New York.
- Teguia, A. and S. Fon Fru, 2007. The growth performances of broiler chickens as affected by diets containing common bean (*Phaseolus vulgaris*) treated by different methods. Trop. Anim. Health Prod., 39: 405-410.