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Effect of yoghurt serum powder dietary supplementation on growth performance and antioxidant status in fattening lambs.

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ABSTRACT.

Large quantities of serum are produced during the strained yoghurt manufacturing process, which is the predominant type of yoghurt in Greece. However, the exploitation of this by-product as an alternative source of energy, protein and mineral elements in animal diets has not yet been examined. Therefore, this experiment was conducted to determine the effects of dietary yoghurt serum supplementation on growth performance and antioxidant status in sheep.

Forty eight male 2 months old lambs of Chios breed were randomly assigned to three experimental groups; control group was fed with a commercial basal diet, whereas the other two groups consumed the same diet, with the only difference that concentrated feed was uniformly supplemented with two levels of yoghurt serum powder (YS1: 25 g/kg feed or YS2: 50 g/kg feed). Lambs were weighed in a weekly basis from the beginning until the end of the experiment and blood samples were collected to measure antioxidant status.

No significant effect of yoghurt serum powder on growth performance of fattening lambs was demonstrated, even after 28 days of dietary supplementation ($P > 0.05$). At the same time, malondialdehyde (MDA) levels in blood plasma were not significantly different among the experimental groups ($P > 0.05$) and no incidents of lambs with diarrhea were recorded. It can be concluded that yoghurt serum powder appears as a promising alternative of the cereals in the diets of fattening lambs, since no negative effects on growth performance and health status were observed.

1 Introduction

Yoghurt production is continually enhanced worldwide; from 19263 tn in 1992 to 61246 tn in 2012, according to FAO. With the growth of the yoghurt manufacturing industry the increasingly larger quantities of yoghurt serum have resulted in greater pollution hazards. Yoghurt serum (may be called whey as well) is mechanically derived after the fermentation of yoghurt and could be a strong environmental pollutant when discharged into streams, since its high organic matter content leads to a high biochemical oxygen demand (BOD5) (Alonso *et al.*, 2010). Although no data exists concerning the use of yoghurt serum, the exploitation of the diverse properties of whey, a by-product of cheese industry with similar composition, in a variety of food systems as source of protein, flavor enhancer, egg white substitute, and food binder for conventional food use is firmly established during the last decades (Walzem *et al.*, 2002). Among others, whey could be an alternative source of energy (high lactose content), nitrogen and mineral elements to cereals in animal diets (Oba, 2011). Whey derived from cheese industry is traditionally used in the diets of pigs, but in areas unsuitable for pig breeding, this by-product could be used in dairy farms as liquid or after water evaporation (Church, 1991). Fermented, ammoniated, condensed whey could be a potential source of dietary non-protein nitrogen (alternative to urea) and could provide carbon skeletons for microbial protein synthesis in sheep (Boukila *et al.*, 1995). However, special care must be taken that the animals do not consume excessive quantities (>20% of dry matter) of dried whey, because it can cause digestive disorders and diarrhea incidents (Anderson *et al.*, 1974).

Since no data exists regarding the utilization of yoghurt serum powder in the diets of fattening lambs, a preliminary study was conducted to examine the possible effects of yoghurt serum powder dietary supplementation on their growth performance and antioxidant status.

2 Materials and Methods

2.1 Experimental Design and diets

Forty eight male 2 months old lambs of Chios breed were used in the present study. The lambs were randomly assigned to three experimental groups; control group was fed with a commercial basal diet (Table 1), whereas the treatment groups consumed the same diet, with the only difference that concentrated feed was uniformly supplemented with two levels of yoghurt serum powder (YS1: 25 g/kg feed or YS2: 50 g/kg feed). Yoghurt serum was mechanically derived after the fermentation of authentic Greek yoghurt with *Lactobacillus bulgaricus* and *Streptococcus thermophiles* in a dry free flowing powder and contained 5.1% protein, 60% lactose, 12.5% galactose, 5.3% lactic acid and 18 g Calcium, 6 g Phosphorus, 6.6 g Sodium, 24.7 g Potassium, 1.7 g Magnesium, 14.4 g Chloride, 0.48 mg Copper, 1.13 mg Ferrum per kg (Hellenic Protein A.E., Athens, Greece).

Lambs were fed in groups (Control or YS1 or YS2) twice daily at 8:00 a.m. and 16:00 p.m. On average, each lamb consumed 550 g of concentrated feed mixture and 500 g of alfalfa hay per day, and the nutritional needs were estimated according to Agricultural and Food Research Council (AFRC, 1993).

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Table 1: Composition and analysis of control diet[1]

Components (g/kg)	**Control**	
Corn	465	
Wheat	120	
Soybean Meal (44%)	210	
Sunflower Meal	50	
Alfalfa Meal	30	
Wheat Bran	40	
Palm (rumen-protected) Oil	25	
Molasses	15	
Sodium Chloride (NaCl)	9	
Calcium Carbonate	18	
Monocalcium Phosphate	14	
Vitamins & Trace elements Premix	42	
Analysis[3]	**Control**	**Alfalfa hay**
Dry Matter – DM (%)	88.0	89.0
Net Energy (MJ/ kg)	7.3	4.0
Crude protein – CP (%)	17.0	17.0
Crude Fiber (%)	5.4	30.0
Ash (%)	8.0	10.0
Fat (%)	5.0	-
Calcium (%)	1.0	-
Phosphorus (%)	0.7	-
Sodium (%)	0.4	

1. Lambs were fed ad libitum; control group was fed with the commercial basal diet, whereas the treatment groups consumed the same diet, with the only difference that feed was uniformly supplemented with two different levels of yoghurt serum powder (YS1: 25 g/kg feed or YS2: 50 g/kg feed)
2. Premix contained per kg: 150 mg Mg, 35 mg Mn, 50 mg Fe, 60 mg Zn, 0.8 mg Se, 0.75 mg Co, 1.25 mg I, 60 mg Se, 200 mg Mo, 15 kIU vitamin A, 2 kIU vitamin D3, 25 mg vitamin E (kIU: 1000 International Units).
3. According to AOAC (1990) and Van Soest *et al.* (1991)

2.2 Growth performance and Antioxidant status

The duration of the experimental period was 28 days and lambs were weighed in a weekly basis from the beginning until the end of the experiment. In order to examine the possible effects of yoghurt serum powder intake on the health status of lambs, blood samples were obtained with minimal disturbance by venipuncture of the jugular vein on day 0, day 7 and day

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28 after the beginning of the experiment. The samples were transferred immediately to heparinized centrifuge tubes and plasma was separated by centrifugation at 4°C within 30 min and was stored at -20°C. The lipid peroxidation values in plasma were determined according to the spectrophotometric method described by Ohkawa *et al.* (1979). Briefly, to each test tube, 0.5 ml of plasma, 0.5 ml of normal saline (0.9% sodium chloride solution), 1 ml of 20% trichloroacetic acid (Merck KGaA, Germany) and 0.25 ml of thiobarbituric acid (TBA) reagent - 200 mg of TBA (Sigma Chemical Co, Germany) in 30 ml distilled water and 30 ml of acetic acid (SDS, France)- were added. The test tubes were kept for boiling at 95 °C for 1 hour. To each of the test tubes, 3 ml of n-butanol (Chem-Lab NV, Belgium) was added and mixed well. The tubes were centrifuged at 3000 rpm for 10 min. The separated butanol layer was collected and read in a spectrophotometer against reagent blank at 535 nm. Thiobarbituric reactive substances (TBARS) content was expressed as nmol of malondialdehyde per ml of plasma. The methods used in the present experiment were in accordance with the national legislation and the guidelines of the Research Ethics Committee of the Department of Animal Science and Aquaculture of the Agricultural University of Athens.

2.3 Statistical analysis

Data referring to body weights (kg) and MDA concentration values (nmol/ml) were analyzed using a mixed model procedure appropriate for repeated measurements per subject, which included nutritional treatment as fixed effect (unstructured covariance structure). All model analyses were performed with SAS/STAT version 9.1.3 (2005).

3 Results

No significant effect of yoghurt serum powder on growth performance (kg) of fattening lambs was demonstrated, even after 28 days of dietary supplementation (24.16, 25.03 and 25.63 for the control, YS1 and YS2, respectively). On the other hand, weight of lambs increased with age, irrespective of experimental group (Table 2).

Malondialdehyde (MDA) levels in plasma were not significantly different among the experimental groups. However, a significant increase in MDA values (nmol/ml) was observed in YS2 group one week after the beginning of the dietary supplementation, an increase that was not so profound after 28 days of yoghurt serum powder dietary supplementation (0.714, 1.131 and 0.917 on day 0, 7 and 28, respectively) (Table 3).

4 Discussion

Although no data exist related to the use of yoghurt serum in animal production, the feeding value of cheese whey products was extensively studied in the United States, especially during the late 1960s and early 1970s. Dried whey derived from cheese industry can be used in small quantities in the diets of fattening lambs (< 6%) without negative effects on growth

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performance, carcass characteristics and health status (Larsen *et al.*, 1963). Boukila *et al.* (1995) reported a beneficial effect of fermented, ammoniated, condensed whey dietary supplementation at the level of 2-3% on feed intake in sheep and suggested that may be a better alternative to urea as a source of non-protein nitrogen. Whey permeate can also be successfully used as feed ingredient (10%) in the diet of highly productive lactating goats, since its inclusion in the diet results in a significant increase of feed intake, possibly due to its high palatability (Rapetti *et al.*, 2002). The higher dry matter intake (+ 9.2%) leads to a significantly higher fat, protein and raw milk production compared to goats fed no whey-supplemented diets (Rapetti *et al.*, 1995).

Table 2. Effect of yoghurt serum powder dietary supplementation on body weights (kg) of lambs (means ± s.e.m.)

Experimental period (days)	Yoghurt powder supplementation (g/kg)			S.E.M.
	0	25	50	
0	20.03	20.41	20.38	0.783
7	21.22	21.56	21.56	0.784
14	22.06	22.56	23.41	0.797
21	23.50	24.37	24.72	0.833
28	24.16	25.03	25.63	0.815

No significant differences were found among the experimental groups (P> 0.05)

Table 3. Effect of yoghurt serum powder dietary supplementation on malondialdehyde (MDA) values (nmol/ml) in blood plasma of fattening lambs (means ± s.e.m.)

Experimental period (days)	Yoghurt powder supplementation (g/kg)			S.E.M.
	0	25	50	
0	0.855^{a}	0.867^{a}	0.714^{a}	0.062
7	1.047^{a}	1.093^{a}	1.131^{b}	0.106
28	0.947^{a}	0.973^{a}	0.917^{c}	0.057

^{abc} Mean values with different superscripts within a column differ (P < 0.001).

No significant differences were found among the experimental groups (P > 0.05)

Schingoethe (1976) also indicated that ruminants can consume dried whey products up to 10% of dietary dry matter in high grain diets without causing digestive disorders or negatively affecting growth and production. The incorporation of dried whey at the rate of 10-65% of the

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concentrated feed or 15% of corn could also increase feed intake in cattle (Galloway *et al.*, 1992; Morrill and Dayton, 1974; Schingoethe and Skyberg, 1981). As it shown by the findings of the present study and the existing literature, yoghurt serum, such as whey, could readily replace a part of the cereals in fattening rations for lambs, without negative implications on their growth.

Malondialdehyde (MDA) levels in plasma were not significantly different among the experimental groups. However, a significant increase in MDA values (nmol/ml) was observed in the lamb group fed with the high level of yoghurt serum, especially one week after the beginning of the dietary supplementation. A possible explanation is that lactic acid in excess could penetrate the wall of the rumen and may cause from slight alterations in some health indices, such as oxidation values in plasma, till serious metabolic disorders, such as acidosis and diarrhea (Anderson, 1975). Lactic acid is produced in the rumen by the breakdown of lactose by bacteria and protozoa and is further metabolized into volatile fatty acids, mainly butyrate.

Feeding trials in cows show that whey dietary supplementation even at the levels of 10-40% of dry matter intake does not have any detrimental effects on animal health, such as digestive disorders and diarrhea incidents (Schingoethe *et al.*, 1980; Pinchasov *et al.*, 1982; Susmel et al, 1995). In the present study, no incidents of lambs with diarrhea were also recorded, possibly as a result of the low dietary supplementation level of yoghurt serum powder. As it is concluded, an adjustment period is therefore necessary to adapt the digestive system to this new form of feed and enable its consumption (Anderson *et al.*, 1974), especially when the concentration of the yoghurt serum powder in the diet increases. In practice, according to the findings of the present study, an adjustment period of about a week with the low level of yoghurt serum powder dietary supplementation (25 g/kg) could alleviate the possible negative effects on animals' health – displayed as increased MDA plasma concentration – caused by increasing the level of supplementation (50 g/kg), at the age of 2- 3 months in lambs.

5 Conclusions

Yoghurt serum powder, such as cheese whey, is a source of energy that could readily replace a part of the cereals in fattening rations for ruminants and enhance the use of non-protein nitrogen by the rumen microflora. It appears as a promising alternative for the diets of fattening lambs, although further experimentation is warranted to elucidate its exact impacts on their metabolism and to evaluate its economical convenience based on the market values of feedstuffs.

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