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Effect of Different Levels of Live Yeast in a High Concentrate Diet on Performance, Blood Constituents and Immune System Status of Zandi Lambs

Research Article

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ABSTRACT

A feeding trial with twenty-seven male weaned Zandi lambs (initial body weight 27.1±0.38 kg) was conducted to evaluate the effect of different levels of *Saccharomyces cerevisiae* SC47 in diet containing high concentrate (85%) on the growth performance, blood parameters and immune system status. Lambs were allocated to one of three treatment diets in a completely randomized design with 3 replicates and 3 observations per replicate including: 1) basal diet without yeast, control diet; (CD) 2) basal diet with 3 g yeast per lambper day, low yeast; (LY) and 3) basal diet supplemented with 4.5 g yeast per lambper day, high yeast; (HY). Regarding dry matter intake (DMI), there was no significant difference among treatments (P>0.05). Average daily gain (ADG) was greater in HY group, but differences among treatments were not significant (P>0.05). Feed conversion ratio (FCR) was not significantly affected by dietary treatments (P>0.05). Differences between concentrations of total protein, globulin and albumin/globulin ratio (A/G) were significant (P<0.05). The highest amount of total protein and globulin was observed in LY group (P>0.05). Lambs in CD group had the highest amount of A/G ratio. No significant differences were found for the hematology results (P>0.05). No significant differences were detected in differential white blood cells, except neutrophil band that was greater in HY group at the twelfth week (P<0.05).It is concluded that the use of dietary live yeast in high concentration can improve performance (P>0.05), plasma biochemical metabolites(P<0.05) and hematological parameters (P>0.05) in Zandi lambs.

KEY WORDS blood constituents, fattening Zandi lambs, high concentrate, immune system, live yeast, performance.

INTRODUCTION

Different feed additives were added to animal's diet with the aim of increase breeding, health and quality of products in animal breeding for a long time. For several years antibiotics were the most important feed additives that was used, but in the last decade, the use of antibiotics in animal husbandry is in question, due to the development of antibiotic resistance. Research shows an association between the use of subtherapeutic dose of antibiotics and antibiotic resistance in organisms (Amabile-Cuevas *et al*. 1995). In an effort to replace antibiotics in animal feeds, many additives have been proposed. Recently, alternatives for substituting these traditional growth promoters have been evaluated,

probiotics, prebiotics and ionophores are examples of these promoters (Heinrichs *et al.* 2003). The probiotics are classically defined as live microbial dietary supplements that when administered through the digestive tract, cause a positive impact on the host's health by improving gut micro flora (FAO, 2002; Salminen *et al.* 1996; Fuller, 1989). Studies on the beneficial impact of probiotics on animal performance have indicated that probiotic supplementation could have positive effects (Miles *et al.* 1981). One of the most common probiotics in ruminants, are *Saccharomyces cerevisiaeyeast* (SC). The use of SC as a probiotic, began during the 1940's and 1950's (Beeson and Perry, 1952). Live yeast consumes free oxygen in the rumen with respiration, so provides an anaerobic environment that proper for rumen metabolic function (Newbold *et al.* 1995). Enzymes, vitamins, saccharides [β-glucans (βGs) and mannan oligosaccharides (MOSs)] found in yeast cell walls have immunomodulative properties. MOSs are capable of neutralizing pathogenic bacteria, and they support βGs in the process of stimulating defense mechanisms (Małaczewska and Milewski, 2010). Other metabolites produced from yeast fermentation may have benefit on performance and health of animals. The main target of using yeast for growing lamb is to increase the breakdown of dietary fiber and protein that lead to increase microbial protein as a main source of amino acids in the small intestinal, consequently, improve growth. Moreover, SC yeast has biologically valuable proteins, vitamin B-complex, important trace minerals and several unique plus factors. Many other beneficial effects identified such as improve performance (Glade and Sist, 1988; Martin *et al.* 1989) and feed efficiency (Onifade and Babatunde, 1996), affects on ruminal pH by reducing activity of lactic acid producer bacteria and thus reduce ruminal acidosis and metabolic disorders (Williams, 1989; Guedes *et al.* 2008; Thrune *et al.* 2009), enhance the immune response (Keyser *et al.* 2007), ability to enhancement of phosphorus availability (Glade and Biesik, 1986; Brake, 1991; Moore *et al.* 1994) and utilization by animals (Erdman, 1989; Pagan, 1990), reduction in cases of disease infection (Line *et al.* 1997). However, the results of using the SC in ruminants are contradictory because of this fact that in many cases no influence or opposing and many unclear results have been shown (Masek *et al.* 2007). The lack of positive results: (Mikulec *et al.* 2010), can be related to biotic factors such as amount and yeast viability and to abiotic factors such as diet sources and animal management (Sales, 2011). One of the most important reasons for such inconsistent results is diet composition. Young animals with high growth potential, need the diets with high protein and energy content according to (NRC, 1985), which can hardly be achieved in an exclusive forage diet. So, to obtain high performance, they should be fed with a high proportion of concentrate diets. Yeasts are most efficient when animals are fed diets overloaded in energy and thus easily fermented by rumen microorganisms (Williams *et al.* 1991) or diets poor in nutrient supply (Jouany *et al.* 1998). Consumption of large amounts of readily fermentable carbohydrates can change the rumen fermentation pattern. This function can increase production of short chain fatty acidsand lactate, and decrease pH, which affect the amounts of cellulolytic bacteria and reduces fiber digestibility and the production of microbial mass (Mackie *et al.* 2002). Therefore, it is necessary to control fermentation and use additives to maintain rumen health and improve animal production. So, the objective of current study was to investigate the effect of different levels of live yeast SC47 in diet containing high concentrate (85%) on the growth performance, blood constituents and immune system status of Zandi lambs.

MATERIALS AND METHODS

Animals, diets and experimental design

Feeding trial with twenty-seven male weaned Zandi lambs (initial body weight 27.1±0.38 kg and 90±5 days-old), that were grouped based on body weight, were conducted to evaluate the effect of different levels of *Saccharomyces cerevisiae* SC47 (biosaf probiotic) in supplemental diet on the growth performance, blood constituents and immune system status. Lambs were grouped based on body weight, ear tagged and vaccinated against internal and external parasites, and were allocated to one of three dietary treatments in a completely randomized design with 3 replicates and 3 observations per replicate including: 1) basal diet without yeast, control diet; (CD) 2) basal diet with 3 g yeast per lambper day, low yeast; (LY) and 3) basal diet supplemented with 4.5 g yeast per lambper day, high yeast; (HY). Basal diet was consisted of commercial concentrate and hays. Ingredients and chemical composition of basal diet according to the dietary nutrient requirements for lambs (NRC, 1985) are provided in Table 1. The lambs were adapted to feed about 2 weeks. During these 2 weeks, feed intake was restricted to 3.5% of body weight (BW), based on the average BW within a pen, to allow animals to adapt to the change in diet and to prevent the occurrence of digestive disorders. After the adaption period to the end of experiments (twelve weeks), they were fed three times a day (7:00 a.m., 13:00 p.m. and 19:00 p.m.) with a total mixed ration (TMR) diet. Each pen had an automatic water cup so they had free access to water all times.

Sampling, measurement and analyses

Food intake was measured daily (before the morning feeding) and DMI was calculated.

Table 1 Ingredients and chemical composition of basal diet

Ingredients	Amount (%)	Chemical composition	
Alfalfa hay[1]	13	Dry matter (%)	88.6
Wheat straw[1]	2	Crude protein (%)	16.6
Barley	60	Total digestible nutrients (%)	77.9
Soybean meal	6	Metabolizable energy (Mcal/kg)	2.81
Wheat	12	Calcium (%)	0.54
Wheat bran	5	Phosphorus (%)	0.32
Limestone	0.5	Potassium (%)	0.89
Mineral and vitamin premix[2]	1	Ca/P ratio	1.68
Sodium bicarbonate	0.5	-	-

[1] Hays approximately chopped into particles of 3 cm.
[2] The mineral-vitamin premix contained per kg: Cu: 500 mg; Zn: 6500 mg; I: 100 mg; Co: 10 mg; Se: 10 mg; Mn: 1000 mg; Fe: 4000 mg; Antioxidant: 12500 mg; vitamin A: 2000000 IU; vitamin D: 220000 IU and vitamin E: 2500 IU.

This value was expressed as grams DMI per day (g/day). Lambs live weight was measured at the beginning of the experiment and every two weeks interval (before the first meal) and ADG was calculated. This value was expressed as grams ADG per day (g/day). Blood samples were collected at the days of 0, 42 and 84, at 10:00 h, through jugular vein (10 mL into sterile tubes containing ethylenediaminetetraacetic acid (EDTA) solution) and analyzed for concentration of plasma biochemical indicators (total protein, albumin, globulin, A/G ratio, blood urea nitrogen (BUN) and cholesterol), hematological parameters [white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), platelets (PLT) and differential white blood cells].

Statistical analysis

Data were analyzed by the general linear model (GLM) procedure of the Statistical Analysis System software (SAS, 1997), in a completely randomized design with three treatments and three replicates. The shapiro-wilk and kolmogorov-smirnov tests were used to confirm normal distribution of data. Initial body weight and the first series of each parameters of concentration of plasma biochemical indicators, hematological parameters (blood cellular elements) and differential white blood cells, were used as a covariant. Means were obtained by LSMEANS procedures and PDIFF was used to compare means. Effects between the control and experimental groups were considered significant when ($P<0.05$) and finally results were presented as least square means with standard error of the means (SEM).

RESULTS AND DISCUSSION

Performance

Least square means and SEM for effect of different levels of yeast supplement on performance of Zandi lambs are presented in Table 2. Considering DMI, there was no significant differences among the dietary treatments ($P>0.05$). So we can conclude that, live yeast (*Saccharomyces cerevisiae* SC47) has no significant effect on feed intake and appetite, and this may explain the lack of effect for the average daily gain and feed conversion ratio, because the dry matter intake and available nutrients concentration in food, determine the amount of nutrients used to meet demands for maintenance and production. In agreement with our results, other authors also did not find any improvement in DMI after yeast addition to lamb diets (Mikulec *et al.* 2010; Hernandez *et al.* 2009).

But in some study an increase in dry matter intake was observed when yeast was fed to bulls (Galina *et al.* 2006) and lambs (Desnoyers *et al.* 2009; Rezaeian, 2004). Average daily gain was greater in HY group compared to the others throughout the experiment, but the differencewas notstatistically significant ($P>0.05$).

Similar to the results of our study, Mandour *et al.* (2009) and Antunovic *et al.* (2006) reported higher, but not significant, ADG for lambs fed diet enriched with probiotics. In contrast, Milewski *et al.* (2009) and Ding *et al.* (2008) reported higher ADG in fatting lambs fed diet enriched with probiotics. Feed conversion ratio was not significantly affected by treatments ($P>0.05$), but at the end of trial, lambs in group HY consistence with ADG had the best total FCR. In Agreement with our results, some of authors also did not find any improvement in FCR after yeast addition to lamb diets (Khalid *et al.* 2011; Mikulec *et al.* 2010). In contrast to our results, Ding *et al.* (2008) and Masek *et al.* (2007) reported better FCR in fatting lambs fed diet enriched with probiotics.

Plasma biochemical indicators

Results of different levels of yeast supplement on concentration of plasma biochemical indicators of Zandi lambs are presented in Table 3. Except for total protein in the twelfth week, for the other periods the highest and lowest amounts of total protein and globulin was observed in LY and CD groups, respectively ($P<0.05$). In general, sub-acute acidosis can cause intestinal inflammation in animals that this situation will have a negative effect on the digestion and absorption, which one of the consequences is impaired digestion and absorption of protein and thus reduction in plasma total protein.

Table 2 Effect of different levels of yeast supplement on performance of Zandi lambs

Item	Treatments			SEM
	Control	Low yeast	High yeast	
Initial weight (kg)	27.35	27.65	26.25	0.379
DMI (g/day)				
First 4 weeks	1185.3	1161.1	1173.9	5.76
Second 4 weeks	1379.0	1361.2	1374.6	4.32
Third 4 weeks	1580.5	1562.0	1573.4	4.49
Total DMI	1381.6	1361.4	1374.0	4.49
ADG (g/day)				
First 4 weeks	183.3	220.8	243.3	15.75
Second 4 weeks	269.5	252.3	278.3	9.84
Third 4 weeks	273.4	261.4	279.7	12.74
Total ADG	242.1	244.8	267.1	10.20
FCR				
First 4 weeks	6.61	6.35	6.14	0.620
Second 4 weeks	5.18	5.80	5.04	0.239
Third 4 weeks	6.46	6.26	5.89	0.361
Total FCR	5.89	5.86	5.33	0.250

DMI: dry matter intake; ADG: average daily gain AND FCR: feed conversion ratio.
SEM: standard error of the means.

Table 3 Effect of different levels of yeast supplement on concentration of plasma biochemical indicators of Zandi lambs

Item	Treatment			SEM
	Control	Low yeast	High yeast	
Sixth week				
Total protein (g/dL)	6.95^{b}	7.57^{a}	7.15^{b}	0.105
Albumin (g/dL)	3.95	3.98	3.93	0.067
Globulin (g/dL)	3.06^{b}	3.63^{a}	3.10^{b}	0.085
A/G ratio	1.31^{a}	1.09^{b}	1.27^{ab}	0.040
BUN (mg/dL)	17.77	16.76	16.62	0.457
Twelfth week				
Total protein (g/dL)	6.25	6.52	6.30	0.132
Albumin (g/dL)	3.73	3.56	3.23	0.098
Globulin (g/dL)	2.48^{b}	2.94^{a}	2.92^{a}	0.079
A/G ratio	1.50^{a}	1.21^{b}	1.11^{b}	0.050
Cholesterol (mg/dL)	60.50	68.83	59.16	2.577
BUN (mg/dL)	18.56	18.30	18.23	0.680

A/G ratio: albumin/globulin and BUN: blood urea nitrogen.
SEM: standard error of the means.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The results showed that the yeast had no significant effect on plasma albumin (P>0.05) concentrations. Lambs in CD group had the highest A/G ratio and BUN, while the lowest amounts of these plasma biochemical indicators were seen in groups supplemented with yeast. Low but not significant concentrations of BUN in response to probiotic supplements can be due to increase ability of the rumen microflora in trapping ammonia (Abo El-Nor and Kholif, 1998). BUN is an indicator of the protein status in ruminants (Sykes, 1978) and its concentration is related to the level of ammonia absorption from the rumen and the deamination of amino acids not deposited in the tissues (Deaville and Galbraith, 1992).

Another possibility for the lower BUN concentration is that additives promote the utilization and deposition of nitrogen in tissues. No significant differences were detected in cholesterol levels between the different groups (P>0.05). Probiotics that can reduce the intestinal pH, could contribute to the regulation of serum cholesterol concentrations by its relationship with bile acids. In the acidic conditions of the intestine, because of the inability to re-absorption of bile acids at the end of intestine, excretion of bile acids is enhanced and therefore serum cholesterol, as a precursor of bile acids, used to rebuild the bile (De Smet *et al.* 1994). Since in the current study *Saccharomyces cerevisiae* SC47 did not reduce the intestinal pH, we did not found any sig-

nificant differences in serum cholesterol levels between the different groups. In agreement with our results, other authors reported similar results for some concentration of plasma biochemical indicators after yeast addition to lamb diets (Khalid *et al.* 2011; Mukhtar *et al.* 2010; Bruno *et al.* 2009; Masek *et al.* 2008).

Hematological parameters and differential WBC

Results of different levels of yeast supplement on concentration of hematological parameters and differential WBC of Zandi lambs are presented in Tables 4 and 5, respectively. Overall no significant effects were recorded on the hematology results (P>0.05). However, the highest amount of WBC was seen in yeast-supplemented groups (P>0.05). There was an increase in values for hemoglobin in LY and HY groups at the twelfth week (P>0.05).

Probiotics can reproduce and develop in the gut wall as a living cell, or can capture antigens released by dead microorganisms, and by different ways, stimulate the immune system and its components (Fuller, 1977). Live yeast did not modify value of RBC and PLT in the experiments and we observed only a slight increase in RBC in group HY at the end of trial (P>0.05). No significant differences were detected in differential white blood cells, except neutrophil band that was greater in HY group at the twelfth week (P<0.05). Probiotics can stimulate and strengthen the immune system by increase of macrophage activity, which this act appears by increasing the ability of phagocytosis of microorganisms. One of this phagocytosis of organisms is neutrophil, that increase in the number of neutrophils band, in response to supplementation of probiotic, can be stimulated macrophage activity (Tizard, 2008; Fuller, 1992).

Table 4 Effect of different levels of yeast supplement on hematological parameter (blood cellular elements) of Zandi lambs

Item	Treatment			SEM
	Control	Low yeast	High yeast	
Sixth week				
WBC (×1/μL)	8474	8556	11393	708.3
RBC (×10^6/μL)	13.65	13.51	13.43	0.285
Hb (g/dL)	11.01	11.04	11.02	0.201
PLT (×10^5/μL)	4.57	3.95	4.41	0.404
Twelfth week				
WBC (×1/μL)	9259	9277	12612	1009.6
RBC (×10^6/μL)	13.72	13.80	14.32	0.288
Hb (g/dL)	10.88	11.39	11.40	0.175
PLT (×10^5/μL)	4.61	4.31	4.62	0.449

WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin and PLT: platelets.
SEM: standard error of the means.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 Effect of different levels of yeast supplement on differential white blood cells of Zandi lambs

Item	Treatment			SEM
	Control	Low yeast	High yeast	
Sixth week				
Neutrophil segmented (%)	33.36	35.12	33.67	1.317
Neutrophil band (%)	0	0	0	0
Lymphocyte (%)	64.54	63.73	64.05	1.303
Abnormal lymphocytes (%)	0	0	0	0
Basophil (%)	0	0	0	0
Monocyte (%)	0.50	0.33	0.33	0.143
Eosinophil (%)	1.66	1	1.66	0.217
PCV (%)	33.50	33.33	32.88	0.704
Twelfth week				
Neutrophil segmented (%)	29.28	32.34	34.53	1.456
Neutrophil band (%)	0^b	0^b	0.50^a	0.090
Lymphocyte (%)	68.99	66.56	63.61	1.477
Abnormal lymphocytes (%)	0	0	0	0
Basophil (%)	0	0	0	0
Monocyte (%)	0.33	0.16	1	0.202
Eosinophil (%)	1.33	0.83	0.50	0.227
PCV (%)	30.42	32.66	32.48	0.573

SEM: standard error of the means.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The immune stimulating effect of SC was ascribed to the activity of βGs and MOs presented in yeast cell walls (Milewski *et al.* 2007). This mechanism involves the stimulation of immune competent cells, mainly by βGs (Xiao *et al.* 2004; Siwicki *et al.* 2004). βGs activate intercellular defense mechanisms where macrophages, T-cells and NK cells play the key role (Demir *et al.* 2007).

The specific ability of MOs to bind selected pathogenic microbes has a profound effect on the organism's health status. MOs blocks microbial lectins and prevent pathogens from colonizing the host's gastrointestinal system (Sharon, 2008). MOs are not degraded by the digestive enzymes of the small intestine, therefore, the attached pathogens are more easily excreted (Spring *et al.* 2000). In this study, where the experiment was conducted, was cleared from contamination and infection and the possibility of create any stress for the lambs was the lowest rate, that it could be one of the factors of lack of response expected for health and immune system. In agreement with our results, Mohamadi and Dabiri (2012) reported that WBC and differential white blood cells (neutrophil, monocyte and lymphocyte) were unaffected by probiotic, prebiotic and synbiotic in Holstein female calves (P>0.05).

Mandour *et al.* (2009) reported that WBC, RBC, neutrophil, lymphocyte, hemoglobin and albumin were unaffected by bio-nutra probiotics in Awassi, Najdi and Najdi crossbred male weanded lambs (P>0.05) and Shim (2005) reported too that, hematological traits (WBC count, neutrophil, monocyte, lymphocyte and hemoglobin) were unaffected by prebiotic, multi-strain probiotic and synbiotic in weaned pigs (P>0.05). In contrast, the results of experiments conducted on suckling lambs (Milewski *et al.* 2009) and cattle (Dobicki *et al.* 2005; Dobicki *et al.* 2007) indicate that yeast preparations have a favorable effect on the animals immune system and values of WBC, Hb and lymphocyte were affected by yeast (P<0.05). Onifade *et al.* (1999) and Onifade (1997) reported a positive correlation between dietary levels of SC with the hematological parameters like WBC, RBC and hematocrit or packed cell volume (PCV) in rabbit and broiler chickens. They suggested that theses correlations may be an additional mechanism growth promotion by supplemental yeast. Recent studies indicated that yeast components may interact with immune systems and triggering immune responses (Muchmore *et al.* 1990; Davis *et al.* 2004).

CONCLUSION

In the present study, the use of live yeast (*Saccharomyces cerevisiae* SC47) in diet containing high concentrate could improve performance (whit not significantly different, (P>0.05)), concentration of plasma biochemical indicators (whit significantly different, (P<0.05)) and hematological parameters (whit not significantly different, (P>0.05)) of Zandi lambs. Greater ADG and better FCR were seen in the group of lambs that fed with 4.5 gr yeast per lambs (HY group), that could probably be due to improved cellulolytic bacteria in the rumen of lambs fed probiotics supplemented diets and reduce the risk of acidosis compared to other groups specially control group, but differences between groups have not been statistically significant (P>0.05). Blood parameters which related to immune system, were unaffected by treatments. This could be due to good hygienic provided to the lambs. The differences in the type and amount of food consumed, type of probiotic or how to use it can be the reason of difference between results of this study and results of other researchers.

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