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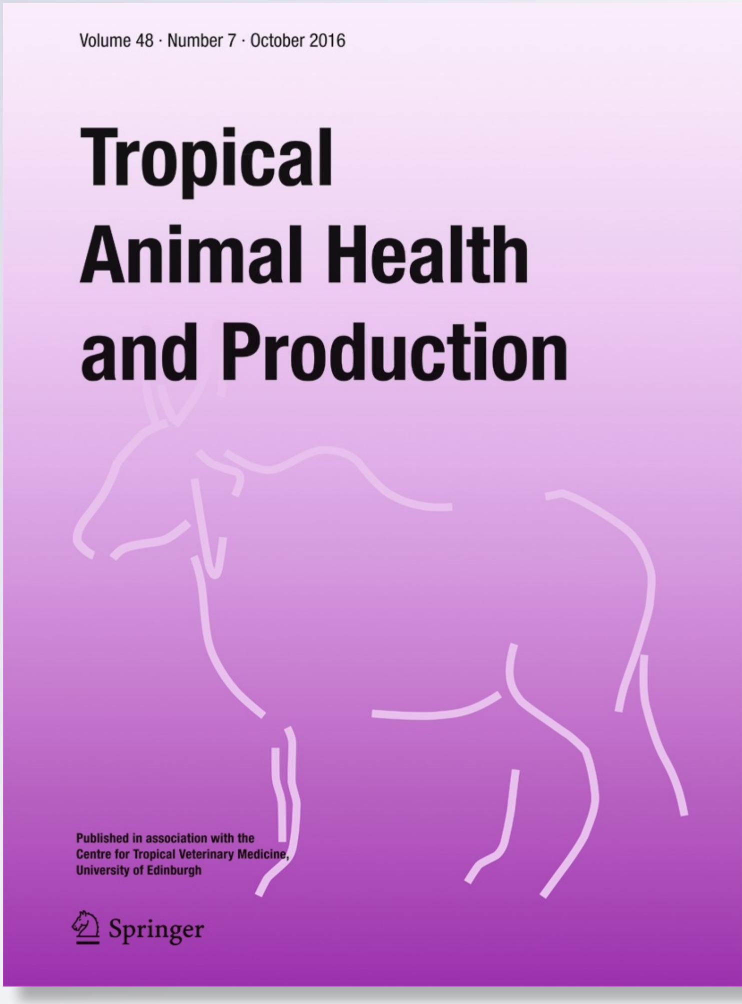

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REGULAR ARTICLES

Fertility, mortality, milk output, and body thermoregulation of growing Hy-Plus rabbits fed on diets supplemented with multi-enzymes preparation

Hany M. Gado[1] · Ahmed E. Kholif[2] · Abdelfattah Z. M. Salem[3] · Mona M. M. Elghandour[3] · Oluwarotimi A. Olafadehan[4] · Maricela A. Martinez[5] · Ahmed Q. Al-Momani[6]

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Abstract The aim of this study was to evaluate the fertility status, milk output, mortality, and body thermoregulation of rabbit does as affected by different levels of multi-enzyme extracts (EZ) in their diets. A total of 120 Hy-Plus rabbit does were divided into four comparable experimental groups (*n* = 30 does per group). Animals of each group were divided in six pens (five animals per pen), and each pen was used as an experimental unit. The first group was kept untreated and fed a commercial diet alone without enzyme extracts (EZ0), while the other groups were fed the same diet but supplemented with 1 (EZ1), 3 (EZ3), and 5 (EZ5) kg/ton of enzyme extracts, respectively. Feeding EZ additive increased ($P < 0.05$) conception and kindling rates, litter size and weight at birth, and litter size and bunny weight at weaning, with decreasing ($P < 0.05$) abortion rate. Moreover, total milk yield increased ($P < 0.05$) with increasing level of enzyme supplementation. Pre-weaning mortality decreased ($P < 0.05$) with EZ inclusion. Signs of vitality (rectal temperature, skin temperature, earlobe temperature, respiration rate, and pulse rate) were improved with EZ inclusion. For all results, 5 kg EZ/ton of feed was more effective than 1 and 3 kg EZ/ton feed. It can be concluded that supplementation of EZ in rabbit diet decreased mortality rate and enhanced fertility status and milk output.

Keywords Enzyme · Fertility · Milk output · Mortality · Rabbits

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Introduction

Feed cost represents about 60–70 % of rabbit keeping costs; therefore, maximizing utilization of nutrients is essential for the profitability and sustainability of rabbit production. Consequently, it has become very necessary to look for locally available, cheap, and nutritionally safe feed additives that would help to cut down production costs and improve production efficiency. Since the European Union banned most of the antibiotic growth promoters in animal nutrition due to cross and multiple resistances, much research has been conducted to explore the use of multi-enzymes as effective substitutes. Enzyme supplementation is meant to complement the endogenous enzymatic capability of animals and increase the nutritive value of feed (Abdel-Aziz et al. 2014, 2015). Recent studies have demonstrated that enzyme addition improves the feed utilization in the small intestine, changing the substrate that arrives at the hindgut. Such changes affect the development of the hindgut microbial population favoring the health status of the animals (Chesson and Steward 2001). The use of enzymes in animal feeds is becoming more common due to lower costs of commercial enzyme preparations, improved enzymes for animal feeds, and a better understanding of the composition of the anti-nutritive compounds (El-Adawy et al. 2013). Enzyme cocktails, more than one enzyme, often improve animal response compared to pure, single enzymes, assuming that cost considerations are not ignored. Several studies have attempted incorporation of exogenous enzymes into rabbit diets to improve nutrients availability; however, in most trials, rabbits appeared less responsive (El-Sagheer and Hassanein 2014). The mode of action of enzymes on the different parts of rabbit gut was addressed by several researchers. Lafond et al. (2015) found that polysaccharide digestion was for 6 h along the digestive tract, using the gastrointestinal model 1 to mimic monogastric metabolism; moreover, their degradation appeared mainly at the jejunal level, and xylanases supplementation increased the amount of reducing end sugars in the jejuno-ileal dialysates. The enzyme extracts used, in the current study, are products containing cellulases, xylanases, α-amylase, and proteases from an anaerobic bacterium and have shown a positive effect on nutrient utilization and performance of ruminant fed low-quality forages (Khattab et al. 2011). Therefore, the objective of the current experiment was to evaluate fertility status, mortality, milk output, and body thermoregulation of growing rabbits fed diets with multi-enzymes extracts as probiotics.

Materials and methods

Experimental work

A total of 120 Hy-Plus rabbit does in four comparable experimental groups ($n = 30$) were used to evaluate the effect of feeding different levels of multi-enzyme extracts (EZ) in diets. Each experimental group was divided into six pens (five animals per pen), and each pen was used as an experimental unit. The first group was kept untreated (control group—EZ0) and fed a commercial diet covering the nutritional requirements of different physiological status of rabbits, while the other three groups were fed the same diet but supplemented with 1 (EZ1), 3 (EZ3), and 5 (EZ5) kg multi-enzyme extracts per ton. The diet was formulated to contain (g/kg dry matter (DM)) clover hay (300), wheat bran (262), soybean meal (160), molasses (30), limestone (10), NaCl (5), and vitamin-mineral premix (5). The following were contained in the vitamin-mineral premix per kilogram: vitamin A (10,000 IU), vitamin D3 (2000 IU), vitamin E (5000 IU), vitamin k (2 IU), vitamin B1 (2 IU), vitamin B4 (4 IU), vitamin B6 (3 IU), vitamin B12 (0.02 IU), biotin (0.2 mg), choline (1200 mg), niacin (40 mg), Zn (60 mg), Cu (0.1 mg), Mn (62 mg), Fe (40 mg), folic acid (1 mg), and pantothenic acid (15 mg). The diet contained (g/kg DM) the following: crude protein 167, ether extract 29.5, crude fiber 131, and digestible energy 10,400 KJ/kg.

The enzyme cocktail was obtained from *Ruminococcus flavefaciens* and characterized for enzyme activities of endoglucanase, α-amylase, protease, and xylanase in addition to the related anaerobic bacteria, which produced these enzymes, coated with starch and glycol. Enzyme activities in the enzyme preparation were determined for xylanase (2.3 U/g), α-amylase (61.5 U/g), endoglucanase (7.1 U/g), and protease (29.2 U/g).

Fertility and milk production experiment

Rabbits of each treatment group were artificially inseminated using the corresponding experimental groups of rabbit bucks. The artificial insemination was carried out as described by Boiti et al. (2005). Palpation of all rabbit does was carried out 12 days post inseminating to determine pregnancy. At kindling, kindling rate, litter size, and weight at birth values were recorded. Pre-weaning mortality rates, bunny weight at weaning, and milk yield and composition per doe were estimated also during the suckling period. Milk yield was estimated after deprivation of the pups from suckling their mothers for 24 h. After this, the pups were weighed before and after suckling, and the increase in pup's weight was taken as the milk yield.

Body thermoregulation

Body thermoregulation was estimated individually in the morning at 8.00 a.m. twice a week, during the experimental period. Both the does ($n = 120$) and the bucks ($n = 192$) were used for this experiment. Rectal temperature was measured by inserting a clinical thermometer in the rectum at a uniform depth of 1.5 cm for one minute. Skin temperature (between neck and loin, medial dorsal surface) was measured from one location on the body surface. The thermometer was fixed on the bare skin and fur, which was combed back into place by finger. Earlobe temperature was measured by a clinical thermometer, which was placed into direct contact with the central area of the auricle. Respiration rate was estimated by the frequency of the flank movements per minute. A hand counter was used to count the flank movement frequencies. Pulse rate was taken by putting the left hand on the left side of abdominal surface of the animal over the heart position and counting the pulse rates for 1 min by a hand counter.

Statistical analyses

Data were subjected to the analysis of variance using the general linear model program of SAS (2001). Data of each pen, used as an experimental unit, were analyzed using the following linear model:

$$Y_{ij} = \mu + B_i + e_{ij}$$

where Y_{ij} = observation on ith breed; μ = overall mean; B_i = effect of ith treated group (i = 1 to 4); and e_{ij} = random error.

Percentage values were transformed to Arc-Sin values to approximate normal distribution before being statistically analyzed. Duncan's new multiple range tests were used to test the significance of the differences among means.

Results

Feeding enzyme additive at different levels did not affect ($P > 0.05$) bunny weight at birth and litter weight at weaning. On the contrary, feeding enzyme increased ($P < 0.05$) conception and kindling rates with increased effect as the enzyme level was increased. The abortion rate was decreased ($P < 0.05$) with feeding enzyme preparation. Moreover, compared to the control rabbits, litter size and weight at birth and litter size and bunny weight at weaning were improved ($P < 0.05$) with feeding enzyme additive, while higher values were observed with increasing enzyme additive levels (Table 1).

During the period from birth to weaning, mean milk yield increased ($P < 0.05$) with the increasing level of enzyme additive compared with the control treatment. Besides, total milk yield during nursing period had the same trend ($P < 0.05$) as mean milk yield (Table 2). Pre-weaning mortality was decreased ($P < 0.05$) with addition of enzyme additive compared with control. Relative to the control treatment, the total pre-weaning mortality rate during suckling period decreased with feeding enzyme additive; moreover, values decreased with increasing enzyme additive levels (Table 2).

Compared with the control rabbits, rabbits receiving diets with enzyme additive supplementation had decreased ($P < 0.05$) means of rectal temperature, skin temperature, ear-lobe temperature, respiration rate, and pulse rate (Table 3).

Discussion

The present results are the second part of another experiment published by Seleem et al. (2014). The results of the published report used in the present discussion could be summarized as increased feed intake (2.8 to 8.5 %), increased daily weight gain (27.0 to 44.8 %), and improved feed conversion ratio (18.8 to 24.7 %). EZ5 had the best results followed by EZ3, then EZ1 and lastly EZ0 (control treatment). Blood parameters (e.g., total protein, albumin, and globulin) were improved with the inclusion of EZ in the diets of rabbits. Improved feed utilization with EZ additive inclusion revealed unaffected taste and smell of the fed diets, as suggested by El-Sagheer and Hassanein (2014).

Improved fertility status (e.g., abortion, conception and kindling rates, litter size and weight at birth and weaning, and bunny weight at birth and weaning) may be related to the improved nutritional status of the mother does. Moreover, EZ additive feeding decreased pre-weaning and total mortalities. The decreased mortality, improved fertility, and nutritional status of the mother does are the results of the stimulatory effect of nutrients made available to the animals, as the improved nutrition enhances productive status of does. Whereas numerous studies have been carried out with ruminants to investigate the potential benefits of feeding EZ (Rojo et al. 2015; Morsy et al. 2016), very few studies have been

Table 1 Fertility traits of rabbit does artificially inseminated by bucks as affected by enzyme additive to diets

Items	Experimental groups[a]			
	EZ0	EZ1	EZ3	EZ5
No. of inseminated does	30	30	30	30
No. of pregnant does	17	21	26	27
No. of kindled does	16	20	26	27
Abortion rate (%)	5.9a ± 0.1	4.8a ± 0.02	0.00b ± 0.0	0.00b ± 0.0
Conception rate (%)	56.7d ± 1.3	70.0c ± 1.5	86.7b ± 1.7	90.0a ± 2.2
Kindling rate (%)	53.3d ± 1.4	66.7c ± 1.1	86.7b ± 1.5	90.0a ± 1.4
Litter size at birth (no.)	6.9 ± 0.9d	7.9 ± 0.9c	9.0 ± 0.8b	9.89 ± 0.8a
Litter weight at birth (g)	276 ± 19c	318 ± 22b	363 ± 21a	396 ± 29a
Bunny weight at birth (g)	40.0 ± 2.8	40.4 ± 1.6	40.5 ± 1.8	40.4 ± 2.2
Litter size at weaning (no.)	6.0 ± 0.6d	7.1 ± 0.5c	8.3 ± 0.4b	9.2 ± 0.5a
Litter weight at weaning (g)	4720 ± 101	5840 ± 84	7130 ± 149	8120 ± 158
Bunny weight at weaning (g)	788 ± 15c	822 ± 16b	857 ± 14a	879 ± 17a

Means bearing different letters within the same row are significantly ($P < 0.05$) different

[a] Diet supplemented with 0 (EZ0), 1 (EZ1), 3 (EZ3), and 5 (EZ5) kg enzyme/ton of diet

Table 2 Milk yield and pre-weaning mortality rate of Hy-Plus rabbits as affected by enzyme additive to diets

Items	Lactation periods (week)	Experimental groups[a]			
	Up to	EE0	EE1	EE3	EE5
Milk yield (g/doe)	First week	512 ± 21c	552 ± 17b	599 ± 21a	622 ± 21a
	Second week	706 ± 24c	759 ± 26b	829 ± 37a	893 ± 355a
	Third week	843 ± 42c	931 ± 38b	1023 ± 40a	1102 ± 44a
	Fourth week	784 ± 28c	841 ± 25b	885 ± 25a	931 ± 29a
Total milk yield during nursing period (g/doe)		2850 ± 62d	3080 ± 97c	3340 ± 102b	3550 ± 108a
Pre-weaning mortality (%)	First week	7.2 ± 0.9a	3.8 ± 0.6b	3.0 ± 0.2c	2.7 ± 0.3c
	Second week	3.92 ± 0.23a	2.63 ± 0.26b	2.21 ± 0.08c	1.56 ± 0.05d
	Third week	2.04 ± 0.04a	2.03 ± 0.05a	1.36 ± 0.03b	1.19 ± 0.03b
	Fourth week	1.04 ± 0.16a	1.38 ± 0.14a	0.92 ± 0.01b	0.40 ± 0.01c
Total pre-weaning mortality rate during suckling period (%)		13.5 ± 1.3a	9.5 ± 1.0b	7.3 ± 1.0c	5.7 ± 0.2d

Means bearing different letters within the same row are significantly ($P < 0.05$) different

[a] Diet supplemented with 0 (EZ0), 1 (EZ1), 3 (EZ3), and 5 (EZ5) kg enzyme complex/ton of diet

carried out on rabbits. However, EZ gained substantial interest in recent years in rabbit nutrition (Abdel-Aziz et al. 2015). Because the large intestine with cecum of the rabbit is a fermentation system similar to the rumen (De Blas and Wiseman 2010; Cunha and Cheeke 2012), some of our explanations would borrow from studies with ruminant animals. The direct results of such actions could have improved the nutrition status of rabbit does receiving the EZ preparation. Improved feed utilization as a result of EZ inclusion reported previously by Seleem et al. (2014) is due to improved digestion rate of fiber fractions and altered fermentation kinetics as well as improved synergism between exogenous and endogenous enzymes (Wang et al. 2001). Feeding EZ additive, in most cases, is paralleled with enhancing effect on microflora growth in gut and cecum and improving total and individual short chain fatty acids and nutrients (e.g., organic matter) digestibility. In the present study, the used EZ preparation contained protease amyloglucosidase xylanases, β—glucanase, cellulase, and hemicellulase.

The decreased mortality of rabbits fed EZ diets is another benefit of EZ additive inclusion. Feeding rabbits on EZ and balanced diets might help to control pre- and post-weaning

Table 3 Body thermoregulation response of rabbits (from weaning to adult age, $n = 78$) as affected by enzyme in diets

		Experimental groups[a]			
		EE0	EE1	EE3	EE5
Rectal temperature (°C)	Males	38.3 ± 0.05	38.1 ± 0.05	38.0 ± 0.03	38.0 ± 0.03
	Females	38.4 ± 0.04	38.1 ± 0.05	38.0 ± 0.04	38.0 ± 0.02
Means ± SE		38.4a ± 0.01	38.1b ± 0.01	38.0c ± 0.01	38.0c ± 0.01
Skin temperature (°C)	Males	37.5 ± 0.03	37.4 ± 0.04	37.3 ± 0.02	37.1 ± 0.02
	Females	37.6 ± 0.04	37.4 ± 0.03	37.3 ± 0.02	37.2 ± 0.02
Means ± SE		37.5a ± 0.02	37.4b ± 0.02	37.3c ± 0.02	37.2d ± 0.01
Earlobe temperature (°C)	Males	37.9 ± 0.06	37.6 ± 0.04	37.4 ± 0.04	37.2 ± 0.05
	Females	38.0 ± 0.04	37.7 ± 0.04	37.4 ± 0.05	37.2 ± 0.02
Means ± SE		38.0a ± 0.03	37.6b ± 0.03	37.4c ± 0.04	37.2d ± 0.03
Respiration rate (rpm)	Males	132 ± 6	120 ± 5	113 ± 5	101 ± 5
	Females	139 ± 6	124 ± 6	115 ± 5	110 ± 5
Means ± SE		135a ± 5	122b ± 4	114b ± 4	106c ± 4
Pulse rate (ppm)	Males	243 ± 10	223 ± 9	216 ± 6	208 ± 6
	Females	247 ± 9	230 ± 7	220 ± 5	210 ± 7
Means ± SE		245a ± 8	227b ± 6	218bc ± 5	209c ± 5

Means bearing different letters within the same row are significantly ($P < 0.05$) different

[a] Diet supplemented with 0 (EZ0), 1 (EZ1), 3 (EZ3), and 5 (EZ5) kg enzyme complex/ton of diet

mortality by limiting pathogen microbial populations (García-Ruiz et al. 2006) or by reduction of N flow at ileum, as observed by García et al. (2005). In an agreement with the present results, Eiben et al. (2004) obtained improved performance and decreased mortality of weaned rabbits with the inclusion of cellulase in their diets.

Milk production improved with enzyme feeding and increased as the enzyme supplementation level increased. As previously shown in the improved fertility status, increased milk output can be explained based on the following factors: (1) increased intestinal metabolic activity, (2) modified intestinal hindgut microbiota by the exclusive competition with intestinal pathogenic bacteria, and (3) modified structure and function of the intestinal epithelium causing stimulated immune system (Malago and Koninkx 2011). Another important factor affecting milk output is the increased litter size. During the lactation, milk output is higher when the litter size is larger (McNitt and Lukefahr 1990). Increased feed intake and utilization are important reasons for increased milk output, as the milk produced by the doe is about 3 to 5 % of the daily feed intake (Coudert et al. 1986).

Higher milk production and the nursing ability of the doe are key aspects for rearing young rabbits. Almost no published reports are available in the literature on the effect of EZ inclusion in diet of rabbits on milk production; therefore, the present results could not be compared. However, higher milk production of EZ supplemented groups may be due to increased availability of nutrients and energy for lactogenesis.

Enzyme additive feeding improved normal rabbit vital signs (e.g., rectal temperature, skin temperature, earlobe temperature, respiration rate, and pulse rate). The normal vital signs depend on recent activity, feed and water consumptions and the physiological stage of the rabbits. The measured physiological traits indicate physiological ability of rabbit body thermoregulation with feeding EZ additive. These results are due to the positive effects of EZ on the respiratory rate and body metabolism.

Conclusions

The exogenous multi-enzymes sourced from anaerobic bacteria and added to the total mixed rations of rabbits enhanced the fertility status, reproductive performance, and milk production and reduced the mortality rate of Hy-Plus rabbits. The 5 kg EZ/ton of feed was the best level compared to other levels (1 and 3 kg EZ/ton feed).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Consent was obtained from all participants included in the study.

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